UNIVERSITY OF ALBERTA

Synthetic Probes of GlcNAcT-V, a Metastasis Associated Glycosyltransferase

BY

Pu-Ping Lu ©

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

DEPARTMENT OF CHEMISTRY

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Dedicated to
my parents, who encourage me,
my wife and daughter, who love me.

ABSTRACT

UDP-GlcNAc: α-Mannoside β-(1→6)-*N*-acetylglucosaminyltransferase (GlcNAcT-V, EC 2.4.1.155) is one of the key enzymes involved in the biosynthesis of highly branched asparagine-linked oligosaccharides. This enzyme has garnered particular interest following observations that its activity increases when cells become transformed. Specific increases in the activity of GlcNAcT-V have also been shown to correlate with the metastatic potential of tumor cells. The trisaccharide octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**5**) is a synthetic acceptor substrate for GlcNAcT-V which adds a β-GlcNAc residue to OH-6 of the central mannose residue.

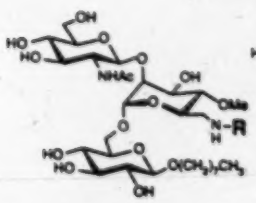
An analog of trisaccharide **5** having both a 4-methoxy and a 6-amino group on the central Man-residue (**11a**) was synthesized in a stepwise fashion by sequential coupling of protected monosaccharide residues. The 6'-amino group in **11a** was then selectively derivatized by either acylation or alkylation with hydrophobic, hydrophilic, charged, aromatic, potential covalently-inactivating and photo-affinity label groups. The eleven trisaccharide analogs (**11a-11k**) thus produced were evaluated for inhibition against GlcNAcT-V isolated from hamster kidney. All of the compounds were competitive inhibitors with K_i values ranging from 21 μM to 297 μM.

The second trisaccharide analog (**12a**), which is deoxygenated at C-6' and carries an amino group on the central Man-residue, was also synthesized by the sequential coupling of three building blocks. The amino trisaccharide **12a** was derivatized using the same methods as were used for preparing the derivatives of **11a**. Compound **12a** and all of its derivatives were shown to be competitive inhibitors of GlcNAcT-V with K_i values ranging from 3-106 μM. The conclusion from enzymatically evaluating these 21 trisaccharide

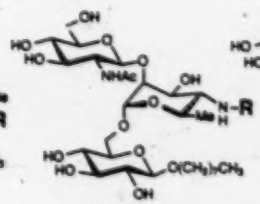
analogs is that in forming the E-I (or E-S) complexes, neither the potentially reactive OH-6' nor the neighboring OH-4', makes important contacts with the enzyme.

Two thioglycosides (**50** and **51**), where sulfur replaces the oxygen atoms in the intersaccharidic linkages, were prepared by multi-step chemical synthesis. Thioglycosides **50** and **51** were kinetically evaluated as substrates for GlcNAcT-V and found to be acceptors with a 2 to 3 fold increase in V_{max} but higher K_m values (**50**, K_m = 376 μM; **51**, K_m = 300 μM) than their parent compound **6** (K_m = 111 μM) which has the natural oxygen linkage. Trisaccharides **50** and **51** were converted into their corresponding tetrasaccharides, by incubation with GlcNAcT-V and UDP-GlcNAc, to verify that the enzyme reacted normally. The results demonstrate that GlcNAcT-V tolerates the substitution of the natural oxygen linkage by sulfur.

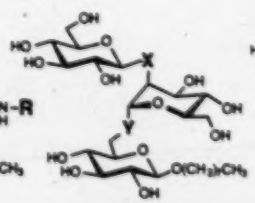
Two stereospecifically labeled trisaccharide acceptors which have 6S - ^{2}H (**5S**) and 6R - ^{2}H (**5R**) were successfully synthesized. Thus, the signals of H-6*pro*R and H-6*pro*S of glucose could be unambiguously assigned. The rotameric distribution about the C5-C6 bond in these two compounds was determined by NMR spectroscopy. Trisaccharide **5** was found to be present in both the gg (60%) and gt (40%) conformations.



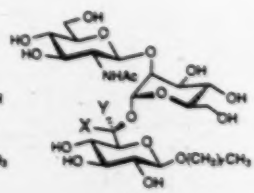
11a-11k
(**11a**: R = H)



12a-12j
(**12a**: R = H)



6: X = Y = O
50: X = S, Y = O
51: X = O, Y = S



5: X = Y = H
5R (6R-^{2}H): X = D, Y = H
5S (6S-^{2}H): X = H, Y = D

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LIST OF ABBREVIATIONS

$[\alpha]$	specific rotation
Ac	acetyl
Ac_2O	acetic anhydride
All	allyl
aq.	aqueous
Asn	asparagine
APT	attached proton test
Ar	aryl
Bn	benzyl
br	broad
BSA	bovine serum albumin
Bu	butyl
Bz	benzoyl
c	concentration (g / 100 mL)
calcd	calculated
CAN	cerium (IV) ammonium nitrate
COSY	correlation spectroscopy
CSA	camphorsulfonic acid
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DMF	*N*, *N*-dimethylformamide
DMP	2,2-dimeoxypropane
dpm	disintegrations per minute

DTPC	2-diazo-3,3,3-trifluoropropanoyl chloride
E.C.	Enzyme Commission
Et	ethyl
FAB	fast atom bombardment
Fuc	fucose
Gal	galactose
GalNAc	2-acetamido-2-deoxy-D-galactopyranose
gem	geminal
Glc	glucose
GlcA	glucuronic acid
GlcNAc	2-acetamido-2-deoxy-D-glucopyranose
GlcNAcT-V	*N*-acetylglucosaminyltransferase-V
GPI	glycosyl-phosphatidyl-inositol
HMQC	inverse 2D ^{13}C-^{1}H heteronuclear multiple quantum coherence
hrs	hours
Hz	hertz
IdoA	idouronic acid
IR	infrared
J	coupling constant
K_i	inhibitory constant
K_m	Michaelis constant
liq.	liquid
m	multiplet
m/z	mass to charge ratio
Man	mannose
Me	methyl
mg	milligram(s)

MHz	megahertz
min	minute(s)
mL	milliliter(s)
mol	mole(s)
mmol	millimole(s)
MS	mass spectrometry or molecular sieve
Ms	methanesulfonyl
NDP	nucleoside diphosphate
NeuAc	*N*-acetyl neuraminic acid, sialic acid
NIS	*N*-iodosuccinimide
NMR	nuclear magnatic resonance
pent	4-pentenyl
Ph	phenyl
Phth	phthaloyl
PMB	*p*-methoxybenzyl or *p*-methoxybenzylidene
pnp	*p*-nitrophenyl
ppm	parts per million
PPTS	pyridinyl *p*-toluenesulfonate
pyr	pyridine
q	quartet
RER	rough endoplasmic reticulum
ROESY	rotating frame nuclear overhauser and exchange spectroscopy
r.t.	room temperature
s	singlet
Sulfo-HSAB	*N*-hydroxysulfosuccimidyl 4-azidobenzoate
t	triplet
Tf	trifluoromethanesulfonyl

TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TOCSY	total correlation spectroscopy
Ts	*p*-toluenesulfonyl
UDP	uridine diphosphate
vic	vicinal
V_{max}	maximum velocity of enzyme-catalyzed reaction
Xyl	xylose

CHAPTER 1

Introduction

1.1 The Biological Roles of Carbohydrates

Carbohydrates exist in nature in the form of free sugars, glycosides, polysaccharides, glycoconjugates or they are integrated into other structures such as DNA. Carbohydrates were long assumed to have only structural and energy-storage functions. However, over the last three decades, a large number of glycoconjugates have been found to play essential roles in many molecular processes in eukaryotic biology and disease [1]. Carbohydrates fulfill important functions in intercellular recognition for both normal and abnormal cellular processes. Examples include conferring blood group specificities, involvement in cell-cell recognition [2], the binding of the sperm to the egg during the fertilization [3,4], guiding neuronal development [5,6], adhesion of viruses and bacteria to their host [7-9], enhancing hormone activities, to name a few. Furthermore, remarkable changes in cell-surface carbohydrates occur with tumor progression which appear to be intimately related to metastasis [10-14]. Recently, carbohydrates have been found to interact with a group of proteins termed selectins [15-18], which are important mediators in the early stages of inflammation. This increased appreciation of the biological significance of carbohydrates has resulted in a resurgence of interest in carbohydrate chemistry. Carbohydrates are sure to have much greater medical importance in the future as current research provides a deeper insight into the biological roles of carbohydrates.

1.2 Naturally Occurring Complex Carbohydrates

Glycoproteins, glycolipids and proteoglycans are the major naturally occurring carbohydrates of animal cells.

Glycoproteins are compounds in which one or several carbohydrate chains are covalently bound to a protein. The carbohydrate moiety may consist of mono-, di, oligo- or polysaccharides. Glycoproteins are divided into two major classes: *N*-linked glycoproteins, where the oligosaccharides are bound to asparagine in the protein (Fig. 1), and *O*-linked glycoproteins, where the oligosaccharides are glycosidically-linked to the hydroxyl groups of serine or threonine [19] (Fig. 2). *N*-linked oligosaccharides are structurally divided into three categories: "high mannose" [20], "complex" [20] and "hybrid" [21] (Fig. 3). The pentasaccharide core, Manα(1→6)[Manα(1→3)]Manβ(1→4)GlcNAcβ(1→4)GlcNAc-Asn, in the boxed area of Fig. 3, is common to all *N*-linked oligosaccharides. Whereas *N*-linked oligosaccharides all share a common pentasaccharide core, *O*-linked oligosaccharides have different core structures [22].

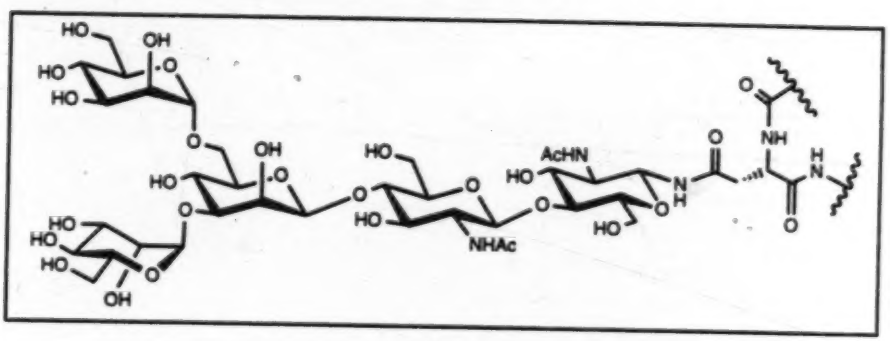


Fig. 1. Core structure of *N*-linked glycoproteins

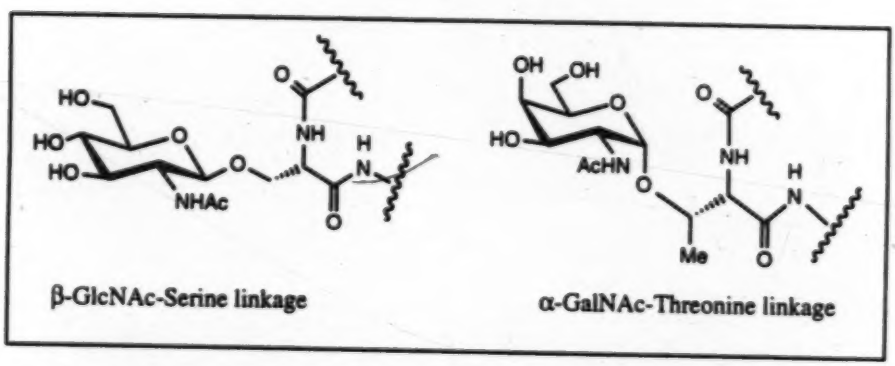


Fig. 2. Two of the likage types present in *O*-linked glycoproteins

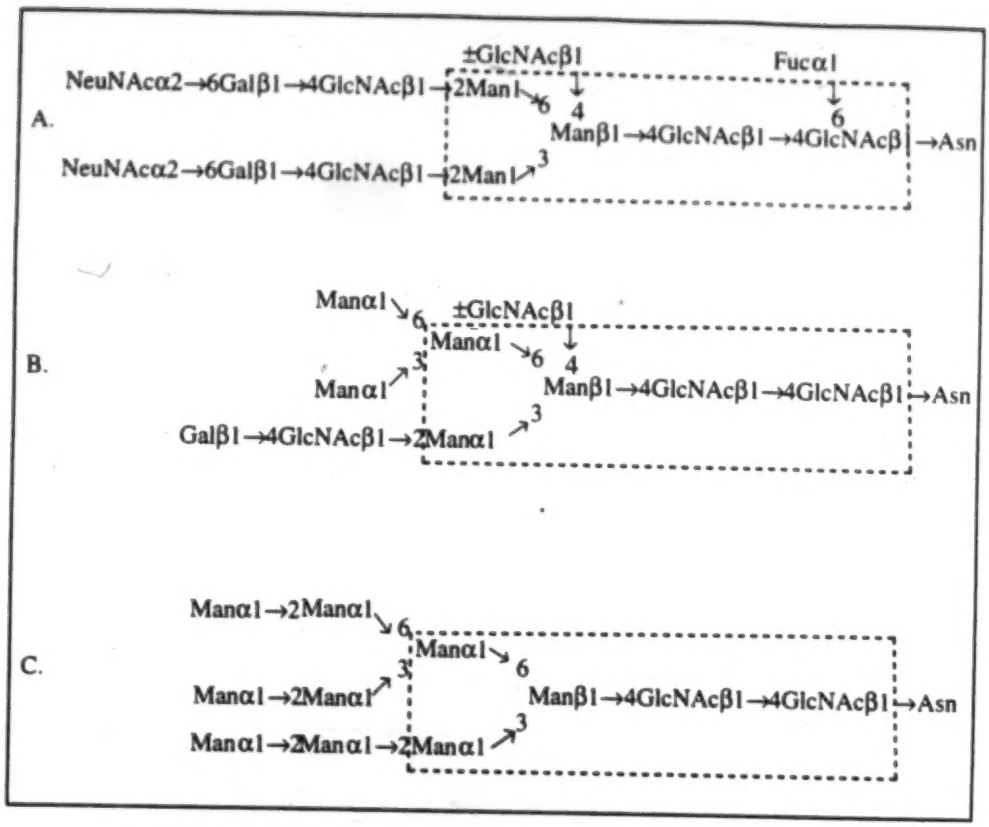


Fig. 3. Three different structural classes of *N*-glycans:

A. complex type; B. hybrid type; C. high-mannose type

Glycolipids are compounds in which the carbohydrates are linked to lipids that are embedded in a cell membrane. Glycolipids can be divided into glycosphingolipids, glycoglycerolipids and glycosyl-phosphatidyl-inositols (GPIs). Glycosphingolipids consist of a carbohydrate moiety *O*-glycosidically linked to a ceramide (*N*-acyl sphingosine) (Fig. 4). Glycosphingolipids can be classified as ganglio-, globo-, lacto-, or neo-lacto- (or muco-) structures according to their carbohydrate structures [23, 24]. Glycoglycerolipids consist of glycerol, fatty acid or fatty ether and carbohydrate (Fig. 5). GPIs are compounds in which the sugar is attached to the phosphate group of glycerol *via* a six membered ring

inositol, while the other two glycerol hydroxyl groups are attached to two long chain alkyl or acyl groups (Fig. 6).

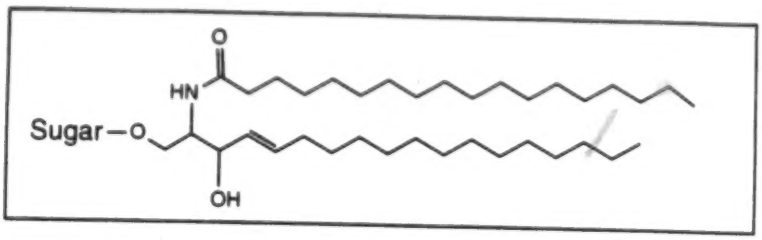


Fig. 4. Glycosphingolipid

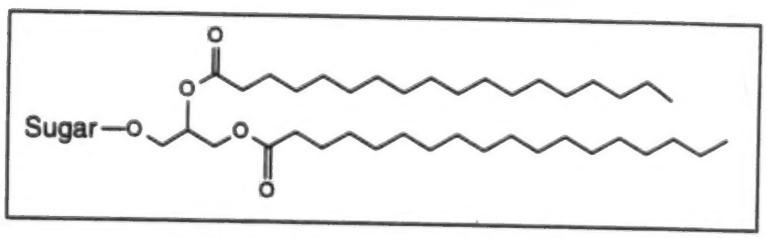


Fig. 5. Glycoglycerolipid

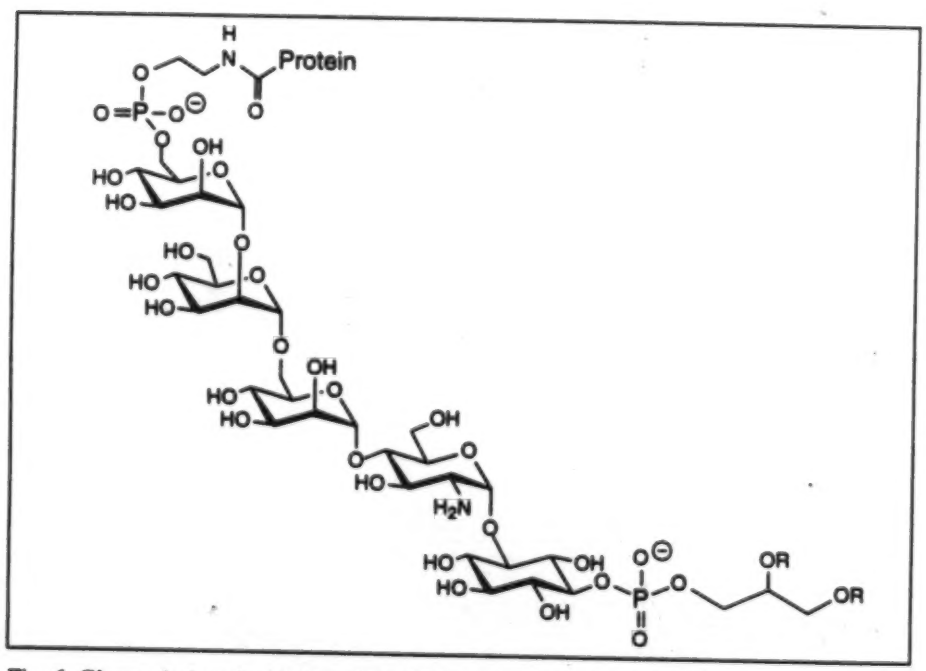


Fig. 6. Glycosyl-phosphatidyl-inositol (GPI) anchors, R = long chain alkyl or ester groups

The third class of complex oligosaccharides are proteoglycans in which polysaccharide chains are made up of unbranched repeating units which are acidic and often additionally sulphated. These are referred to as glycosaminoglycans (GAG) (Fig. 7). Heparin is a well-known GAG. Proteoglycans are found mainly in connective tissues.

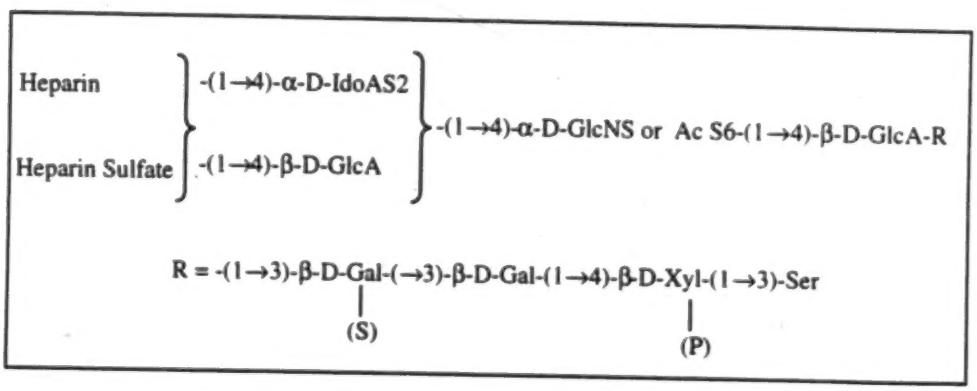


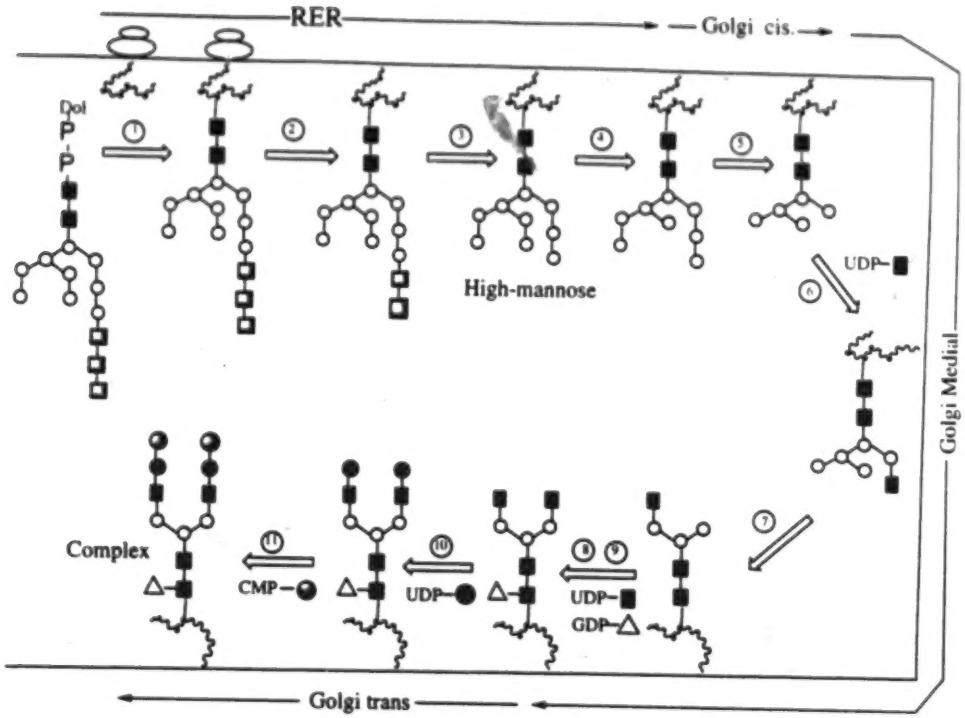
Fig. 7. Proteoglycans. S = sulfate, P = phosphate

1.3 Biosynthesis of Complex Carbohydrates

The biosynthesis and processing of complex carbohydrates are different from the biosynthesis of nucleic acids and proteins. Nucleic acids and proteins are linear molecules in which the bonds between the individual monomers are the same. The biosynthesis of nucleic acids and proteins is therefore efficiently controlled by a template-directed mechanism. Carbohydrates are unique in the complexity of their structures. First, the linkage between two sugars creates two diastereomers, termed α- or β-anomers. Second, a sugar residue can link to three or four different hydroxyl groups on neighboring sugar residues allowing oligosaccharides to have branches. The assembly of complex carbohydrates can thus not be template-directed and requires an elaborate assembly line. The biosynthesis of complex carbohydrates involves two classes of enzymes, glycosidases and glycosyltransferases, which have complementary functions. Glycosidases [25, 26],

which are responsible for the cleavage of glycosidic bonds, can be classified into catabolic enzymes and trimming enzymes. Catabolic glycosidases are involved in the breakdown of ingested oligo- and polysaccharides, or carbohydrate chains of glycoproteins and glycolipids, while trimming glycosidases are involved in the processing of carbohydrate chains during glycoprotein biosynthesis. Glycosyltransferases catalyze the transfer of a monosaccharide from a sugar-nucleotide donor to the hydroxyl group of saccharide acceptors [19, 27-33].

Although the precise pathways and regulatory mechanisms for the biosynthesis of glycoproteins are still unknown, some general features of the process are now recognized [34, 35]. For asparagine-linked (*N*-linked) oligosaccharides (Fig. 8), the first step in the biosynthesis is the transfer of lipid linked high-mannose oligosaccharide $Glc_3Man_9GlcNAc_2$-dolichol pyrophosphate (Dol-P) ***en bloc*** to an asparagine residue in a polypeptide chain by a protein-oligosaccharyl transferase [19]. The lipid-linked high-mannose oligosaccharide is synthesized by addition of one GlcNAc, nine mannose and three glucose units to Dol-PP-GlcNAc, which is formed by the transfer of GlcNAc-1-P from UDP-GlcNAc to the membrane lipid dolichol phosphate [36]. During the transfer from the rough endoplasmic reticulum (RER) to the Golgi apparatus, the structure of the precursor is modified by processing such as trimming of sugars by glycosidases and the addition of sugars by glycosyltransferases. While still in the RER, glucosidase I specifically removes the terminal α-1,2-glucose moiety (reaction 2), and then glucosidase II removes two α-1,3-glucose residues (reaction 3). α-1,2-Mannosidases catalyze the removal of at least one α-1,2-mannose moiety (reaction 4), and the remaining structure is transported in vesicles to the cis face of the Golgi apparatus. Once inside the Golgi, the glycoproteins are processed according to their final destination in the cell. One key pathway is the removal of three mannose residues by Golgi mannosidase I (reaction 5) followed by the addition of a GlcNAc residue by *N*-acetylglucosaminyltransferase I (GlcNAc T-I)



① Oligosaccharyltransferase, ② α-glucosidase I, ③ α-glucosidase II,

④ ER α1,2-mannosidase, ⑤ Golgi α-mannosidase I ⑥ *N*-acetylglucosaminyltransferase I,

⑦ Golgi α-mannosidase II, ⑧ *N*-acetylglucosaminyltransferase II, ⑨ fucosyltransferase

⑩ galactosyltransferase, ⑪ sialyltransferase

■ : *N*-Acetylglucosamine, ○ : mannose, ◘ : glucose, △ : fucose

● : galactose, ◒ : sialic acid

Fig. 8. Schematic pathway of *N*-linked oligosaccharide biosynthesis and processing [34]

(reaction 6). This addition controls the processing of high mannose *N*-glycans to hybrid and complex *N*-glycans (Fig. 3) [28]. Then, α-1,3- and α-1,6-mannose residues are removed by mannosidase II to create an intermediate oligosaccharide (reaction 7). The completed sugar chains are then synthesized by the sequential reaction of glycosyltransferases such as *N*-acetylglucosaminyltransferase II, fucosyltransferases, galactosyltransferases and sialyltransferases. The newly synthesized glycoproteins are then transferred to their final destination in the cell or on the cell surface where the carbohydrate chains face the outside.

The synthetic pathway for *O*-linked oligosaccharides differs from that of *N*-linked oligosaccharides in that there is no transfer of a large oligosaccharide from lipid carrier to protein, nor is there processing by the action of glycosidases. The *O*-linked oligosaccharides are built up by the successive addition of individual monosaccharide units catalyzed by glycosyltransferases [37]. Similarly, ceramide based glycolipids are synthesized by the sequential action of glycosyltransferases in the Golgi apparatus, building up the carbohydrate chains one monosaccharide unit at a time [38].

1.4 Glycosyltransferases

Glycosyltransferases [19, 27-33] transfer a monosaccharide from activated carbohydrate donors (sugar-nucleotide) to the hydroxyl groups of a saccharide, or a saccharide linked to an aglycon (i.e. protein or lipid), to form a new glycosidic bond (Fig. 9). Glycosyltransferases are generally characterized by having a precise specificity for both the acceptor and sugar-nucleotide substrate. These enzymes are found mainly in the Golgi apparatus. According to the rule, 'one linkage-one glycosyltransferase', the existence of a large number of complex oligosaccharide structures implies the existence of well over 150 different glycosyltransferases. Many glycosyltransferases have been cloned to date [39],

but no crystal structures have yet been reported. The most common sugar-nucleotide donors in mammalian metabolism are UDP-D-glucose (UDP-Glc), UDP-N-acetyl-D-glucosamine (UDP-GlcNAc), UDP-D-galactose (UDP-Gal), UDP-*N*-acetyl-D-galactosamine (UDP-GalNAc), UDP-D-xylose (UDP-Xyl), UDP-D-glucuronic acid (UDP-GlcA), GDP-L-fucose (GDP-Fuc), GDP-mannose (GDP-Man), and CMP-sialic acid [27].

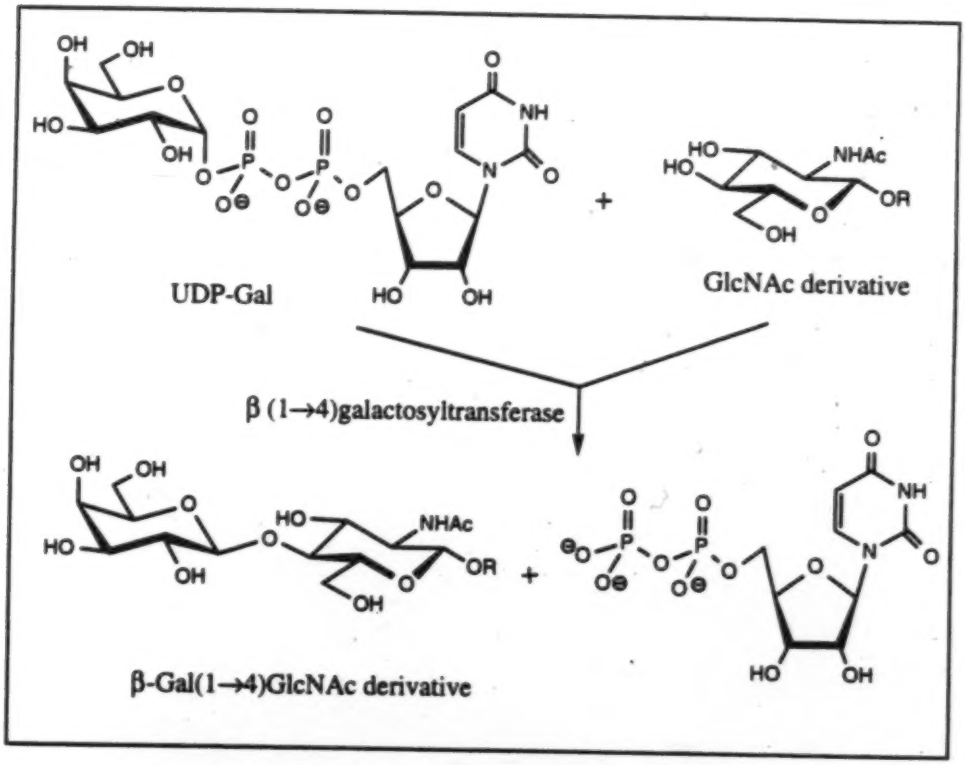


Fig. 9. A glycosylation reaction catalyzed by a glycosyltransferase

The glycosyltransferases are usually membrane-bound and detergent treatment of the membrane preparations is required for solubilization and full expression of enzymatic activity *in vitro* [40]. Most of the transferase assays require addition of exogenous divalent cations (usually Mn^{2+}).

Although the precise mechanisms of glycosyltransferase reactions are unknown, some general features of the mechanisms are now accepted. Fig. 10 schematically depicts the active site of a typical glycosyltransferase [41]. Since glycosyltransferases are specific for both donor and acceptor, there must be at least two major binding domains, one for the activated glycosyl donor and another for elongating the acceptor. Usually, there is a third domain that binds a divalent cation, which probably participates in glycosyl activation through ligation and consequent stabilization of the pyrophosphate moiety [41]. The mechanism of the glycosyltransferse-catalyzed reaction involves nucleophilic substitution by the acceptor on the anomeric carbon of the donor. Since the hydroxyl group is not highly nucleophilic, it has been suggested that a basic residue in the active site activates the hydroxyl group [42]. It was further postulated that metal ions and/or protons serve as promoters for the cleavage of the nucleoside diphosphate (NDP) group [43, 44].

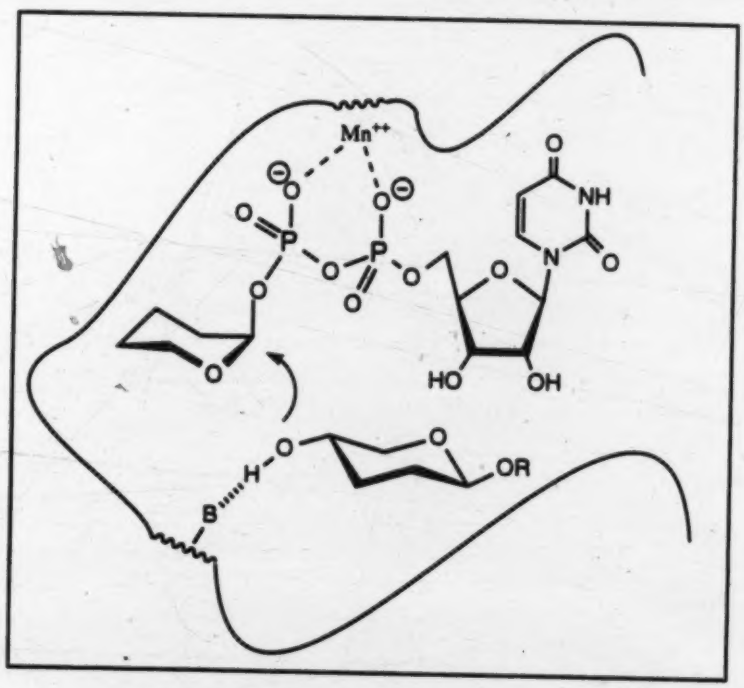


Fig. 10. Schematic of a glycosyltransferase active site

The mechanism of the glycosyltransferase-catalyzed reaction has been investigated using positional isotope exchange, α-secondary deuterium isotope effects, and inhibition studies with potential transition state analogs. It was proposed that the transition-state structure of the glycosyltransfer reactions has a flattened half-chair conformation with substantial oxocarbenium ion character at the anomeric position [45, 46], similar to that of glycosidase-catalyzed reactions [26]. Recently, C.-H. Wong proposed a mechanism for human α-1,3-fucosyltransferase V reaction, based on isotope effect and inhibition studies, where the glycosidic cleavage occurs prior to the nucleophilic attack, in a process between S_N1 and S_N2 reactions [47-49] (Fig. 11.).

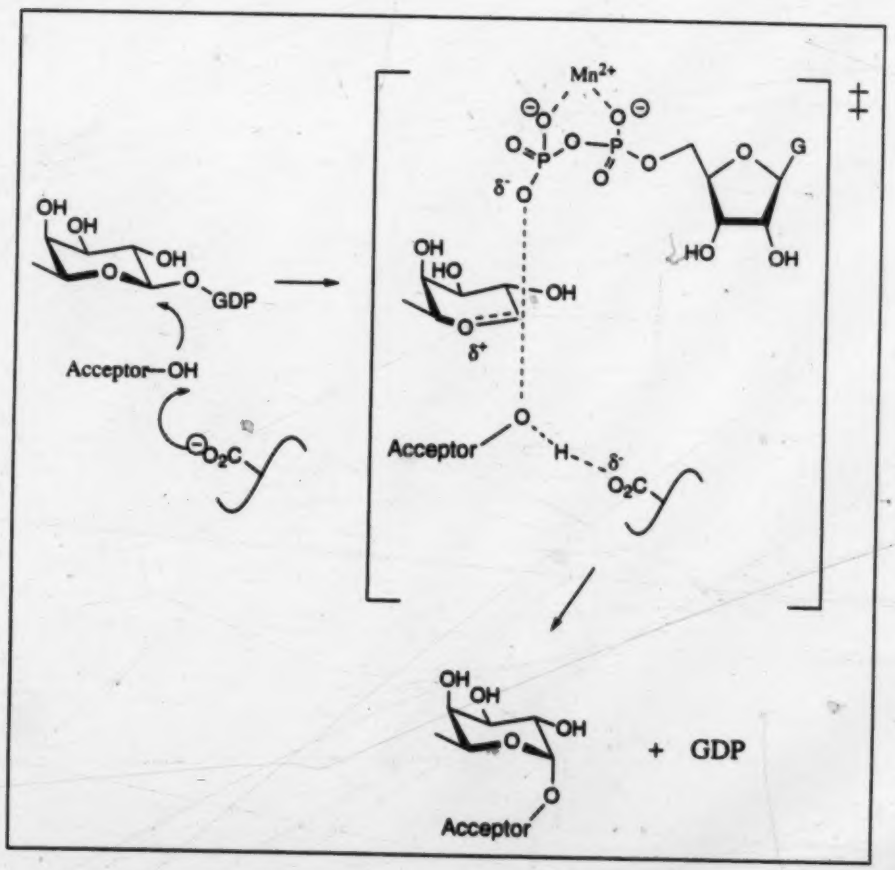


Fig. 11. Proposed mechanism for human α-1,3-fucosyltransferase V

1.5 Inhibitors of Glycosyltransferases

Because of the biological importance of cell-surface oligosaccharides, glycosylation inhibitors have been the target of intensive investigation in biology, chemistry and pharmaceutical science [26, 34, 50]. However, most of the studies involving inhibition of glycosylation have been limited to the inhibition of either the enzyme that transfers the carbohydrate from the dolichol-carbohydrate precursor to the protein [51, 52] or the glycosidases that act in the early stage of biosynthesis of *N*-linked oligosaccharides [26]. Such inhibitions result in global *N*-glycosylation patterns that are drastically different from those that occur naturally on cells but have no direct effect on *O*-linked oligosaccharides and glycolipids.

The development of specific inhibitors for the Golgi-localized glycosyltransferases which control the biosynthesis of the terminal carbohydrate structures has been severely hampered due to the two main factors: the difficulty in the synthesis of natural substrates (often relatively large oligosaccharides) and the lack of knowledge of substrate specificity. Since Lemieux discovered that proteins were not likely to require carbohydrates much larger than a trisaccharide for faithful recognition, based on studies of the binding of oligosaccharides by monoclonal antibodies and lectins [53-56], smaller oligosaccharide fragments have been examined as glycosyltransferase substrates [57]. The use of smaller synthetic substrates makes the probing of substrate specificities of the enzymes much simpler.

Glycosyltransferase inhibitors can in principle not only alter the biosynthetic pathway of *N*-linked oligosaccharides, but also perturb the biosynthesis of both *O*-linked glycoproteins and glycolipids. Glycosyltransferase inhibitors may provide tools for studying the effects of modified cell-surface glycosylation.

1.6 *N*-Acetylglucosaminyltransferases

There are at least 10 different *N*-acetylglucosaminyltransferases known to be involved in the biosynthesis of the asparagine-linked oligosaccharides. The sites of glycosylation of the six branching enzyme are shown in Fig. 12 [58-61]. All of these enzymes use UDP-GlcNAc as the glycosyl donors, however, they are different in their specificity for their acceptor structures. Of the *N*-acetylglucosaminyltransferases which are involved in the biosynthesis of *N*-glycans, the genes for GlcNAc T-I, II, III and V have now been cloned [62]. In addition, two other GlcNAc transferase genes, GnT-I (a branching enzyme) and core 2 GnT (involved in *O*-glycan biosynthesis) have also been cloned [62].

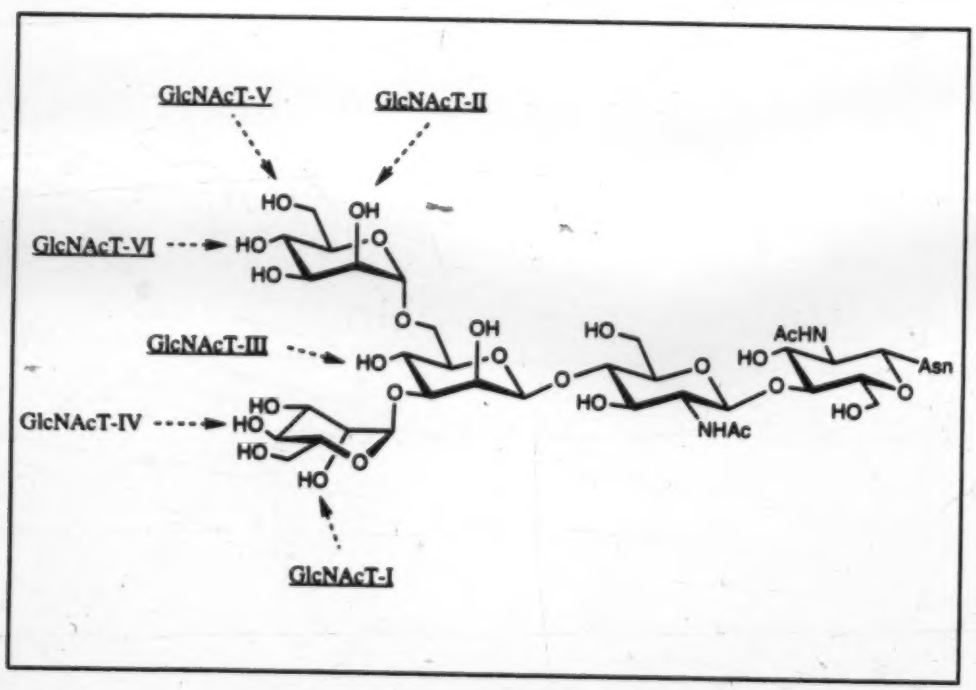


Fig. 12. Sites of glycosylation by GlcNAcT-I to VI

1.7 *N*-Acetylglucosaminyltransferase-V

UDP-GlcNAc: α-Mannoside β-(1→6)-N-acetylglucosaminyltransferase (GlcNAc T-V) is one of the key enzymes involved in the biosynthesis of highly-branched asparagine-linked oligosaccharides [63, 64]. This enzyme transfers GlcNAc from UDP-GlcNAc to the α-1,6-linked mannose of the trimannosyl core of biantennary and triantennary *N*-linked oligosaccharides. After galactosylation, this transfer provides the preferred branch for the subsequent "i" GlcNAc-transferase that initiates the synthesis of long polylactosamine chains [65, 66]. The result is that a single addition by GlcNAcT-V can result in a disproportionate increase in cell surface glycosylation [67-69]. The product polylactosamine chains can be substrates for several other glycosyltransferases which add fucose and sialic acid as described in Fig. 13. The final structures (LeX, sialyl-LeX, poly LeX etc.) are common tumor antigens [70]. These oligosaccharides may contribute to tumor metastasis by a number of mechanisms, which include interfering with cell-cell and cell-substrate adhesion, e.g. *via* integrin receptors or acting as adhesion molecules for receptors (selectins) on the epithelium, allowing extravasation (removal from blood circulation) and thereby the formation of new tumor colonies [69-71].

The expression of GlcNAcT-V activity is regulated in a number of cellular systems [72]. GlcNAcT-V levels vary remarkably in different mouse tissues [73]. This enzyme has attracted considerable interest as a potential tumor marker. It has been found that GlcNAcT-V levels are elevated when the cells are transformed by tumor viruses [67, 74] and several oncogenes [75-77], while the levels of other glycosyltransferases remain unchanged. Specific increases in the activity of GlcNAcT-V have also been shown to correlate with the metastatic potential of several tumor cell lines [69, 73, 78-80], while reduced β-1,6 branching results in lowered metastatic potential [78]. More recently, the activity of

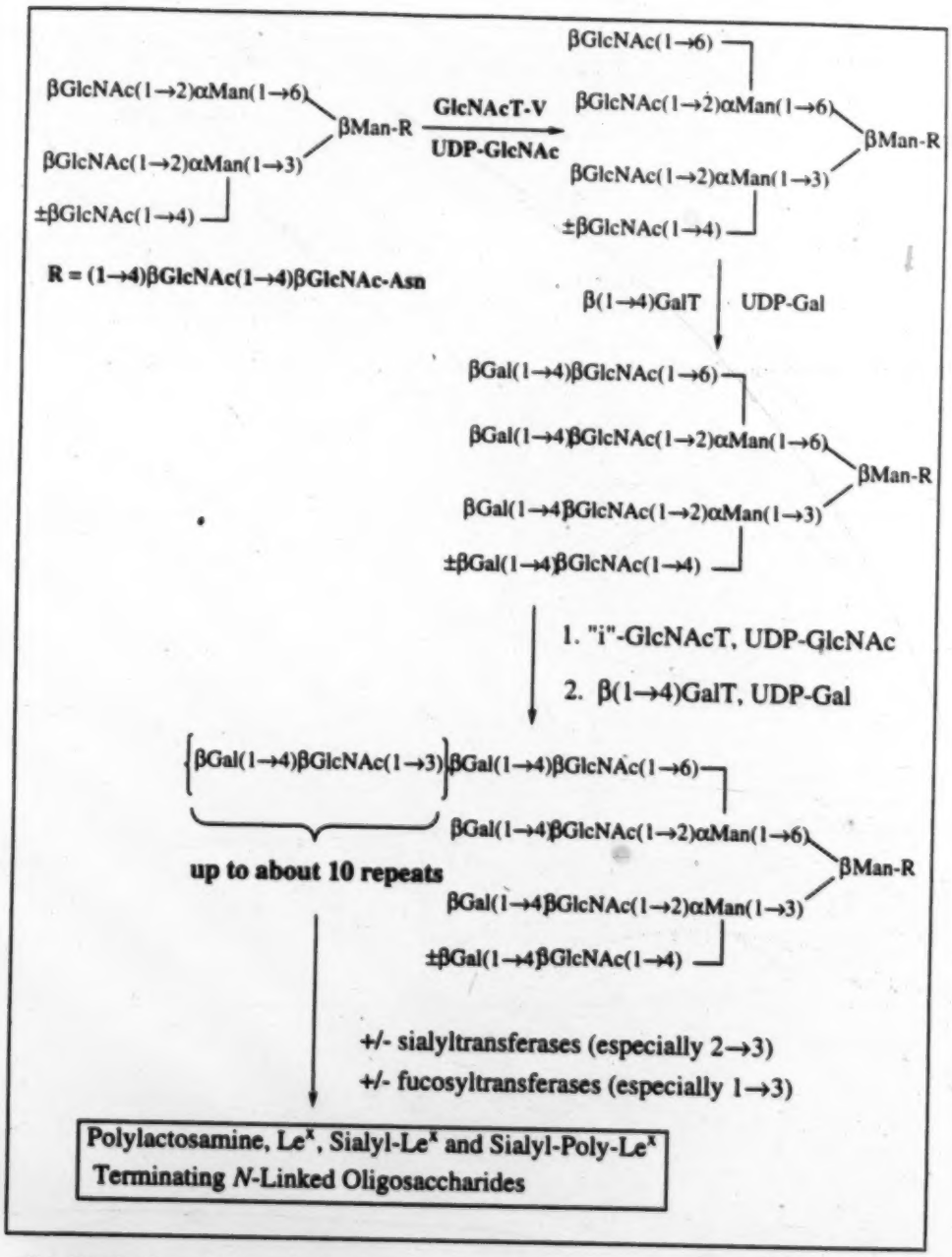


Fig. 13. Preferred biosynthetic pathway for the formation of polylactosamine chains and tumor-associated antigens.

GlcNAcT-V has been found to correlate with cellular proliferation. In HepG2 cells, GlcNAcT-V activity is decreased when the cells become confluent and the growth rate declines [81]. In T lymphocytes, the enzyme activity increases as the cells are activated and stimulated to proliferate [82].

Due to its biological importance and its involvement in cancer metastasis, GlcNAcT-V has become the subject of extensive investigation.

1.8 Cloning of *N*-Acetylglucosaminyltransferase-V

M. Pierce's group purified GlcNAcT-V from rat kidney by sequential affinity chromatography using first UDP-hexanolamine-agarose and then a synthetic trisaccharide inhibitor-agarose column [83]. They isolated a cDNA that contains the entire coding sequence for GlcNAcT-V. The sequence encodes a type II membrane protein of 740 amino acids. The clone was then expressed in COS-7 cells [84].

N. Taniguchi's group obtained a highly purified GlcNAcT-V from human serum-free conditioned medium in which human lung cancer cells were grown [85]. Using designed amino acid sequence oligonucleotide primers, a human GlcNAcT-V cDNA clone was then obtained from a human fetal library [86]. The sequence homology between the human and rat GlcNAcT-V was 97% at the amino acid level [66].

1.9 Synthetic Probes of *N*-Acetylglucosaminyltransferase-V Specificity

A. Work from the University of Alberta Groups

Biosynthetically, glycopeptides bearing the minimum heptasaccharide sequence **1** are the natural substrates for GlcNAcT-V. GlcNAcT-V transfers a GlcNAc unit to OH-6 of the 1,6-linked mannose residue, producing the additionally-branched octasaccharide **2** (Fig. 14) [63, 64]. In 1986, Tahir and Hindsgaul reported the first synthetic trisaccharide **3** that was an effective acceptor substrate for GlcNAcT-V [57]. Trisaccharide acceptor **3**, which is a partial structure of **1**, was shown to yield the expected tetrasaccharide **4** on reaction with GlcNAcT-V [87]. The enzyme tolerates the substitution of the natural β-Man residue in **3** by a β-Glc residue, since trisaccharide **5** was later shown to be an excellent substrate [88]. It was also found that the enzyme further tolerates the substitution of the β-GlcNAc residue by a β-Glc moiety, since the deaminated trisaccharide **6** was found to be an acceptor for GlcNAcT-V [89]. In the synthetic trisaccharide acceptors (**3**, **5** and **6**) (Fig. 14), hydrophobic aliphatic aglycons were incorporated into the structures to facilitate the enzyme assays by using the C-18 "Sep-Pak" procedures [90].

Synthetic trisaccharide acceptor **5** is known [139] to be conformationally labile around the α-Man(1→6) linkage and exists as a mixture of the rapidly interconverting "gg" and "gt" rotamers. In order to check whether GlcNAcT-V recognized **5** in a specific conformation, two conformationally restricted trisaccharides **7** (gt) and **8** (gg) (Fig. 15), where the possibility for rotation about the C5-C6 bond of the β-Glc residue has been eliminated by linking O-4 and C-6 with an ethylene bridge, were successfully synthesized and enzymatically tested [91]. Enzymatic results indicated that the gg rotamer **8** is preferred (227% efficiency), while rotamer **7** (gt) was poorly recognized.

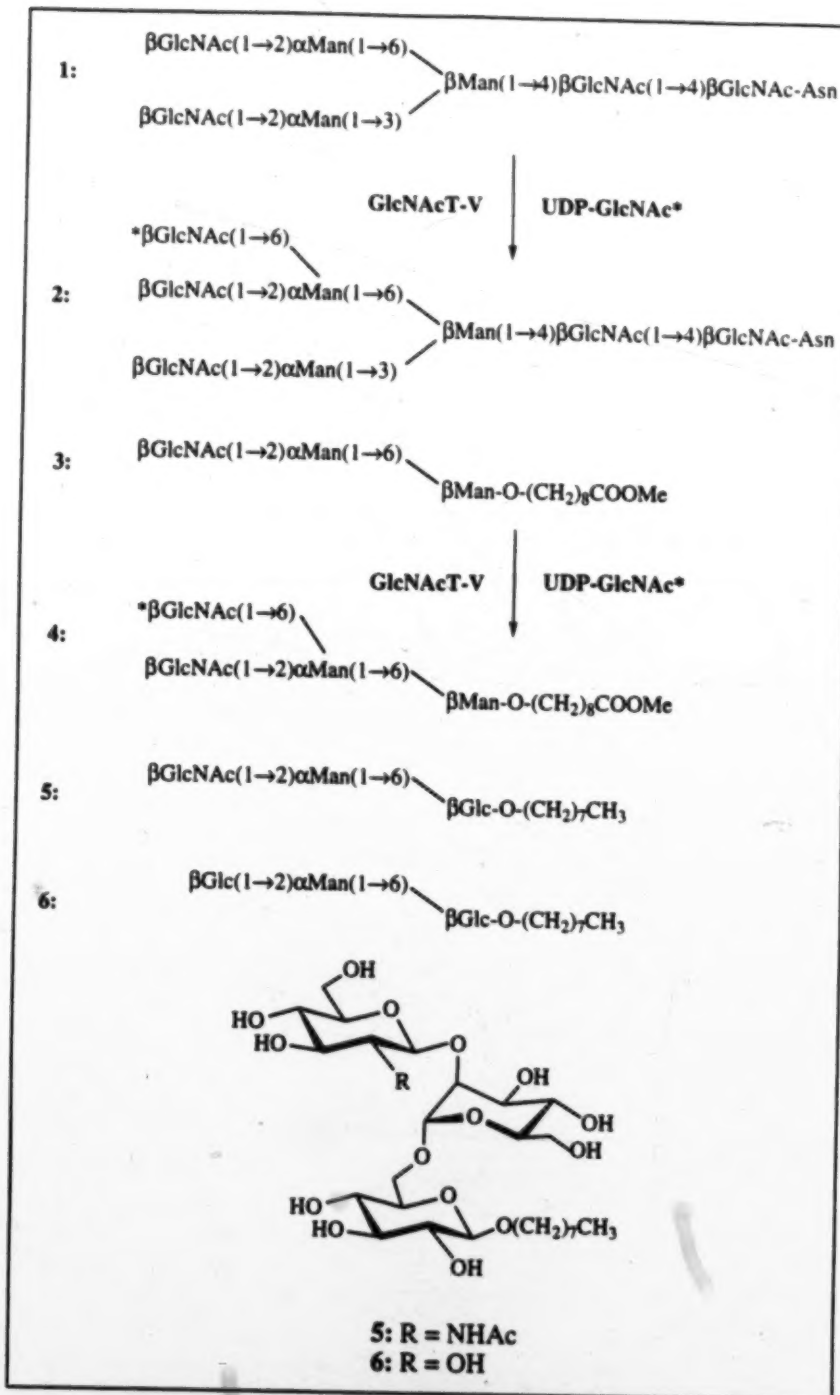


Fig. 14. Glycosylation reactions catalyzed by GlcNAcT-V and the structures of synthetic acceptors for GlcNAcT-V

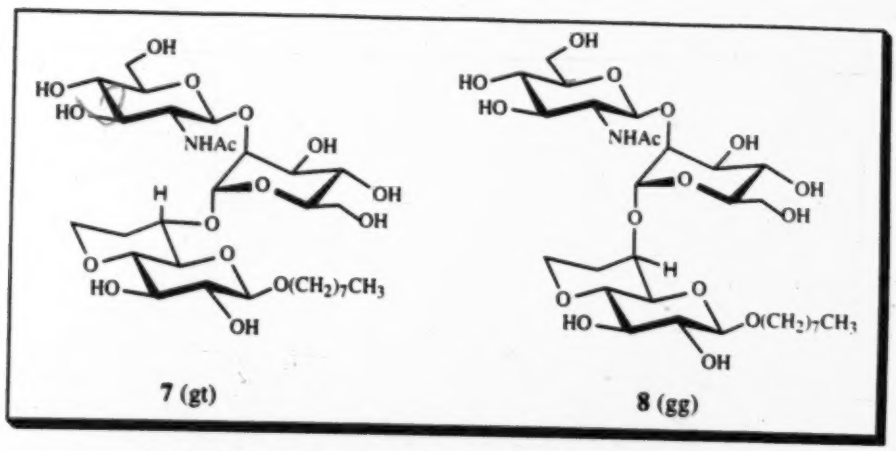


Fig. 15. Two conformationally restricted trisaccharide analogs

The role of the central mannose residue of **5** in substrate recognition by GlcNAcT-V has been investigated. The 3'-*O*-methyl analog of **5** was found to be an excellent acceptor substrate with a slightly higher K_m* than **5** [92]. The analogs where the reactive OH-6 of the mannose residue were removed (6-deoxy, 6-*O*-methyl, or 6-deoxy-6-fluoro) are still recognized and bound by the enzyme and were all found to be competitive inhibitors with K_i values in the range 24-70 μM [92]. The 6'-deoxy-analog (**9**) (Fig. 16) of **5** was the first inhibitor specific for a glycosyltransferase and had a K_i in the range 30-70 μM depending on the source of the enzyme [93]. Modification of OH-4 of the central mannose residue did not affect the recognition by GlcNAcT-V. The 4'-deoxy analog was found to be an excellent acceptor of GlcNAcT-V, while the 4'-*O*-methyl derivative (**10**) (Fig. 16), which retains the potentially-reactive OH-6, was found to be a competitive inhibitor (K_i = 14 μM). It was therefore rationalized that the 4'-*O*-methyl group does not affect the binding but sterically precludes the transfer reaction [94]. It was concluded that none of the OH groups on the central mannose residue are critical for the recognition by the enzyme.

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* The definitions of K_m, V_{max}, K_i and the equations used to determine them are presented in the Appendix.

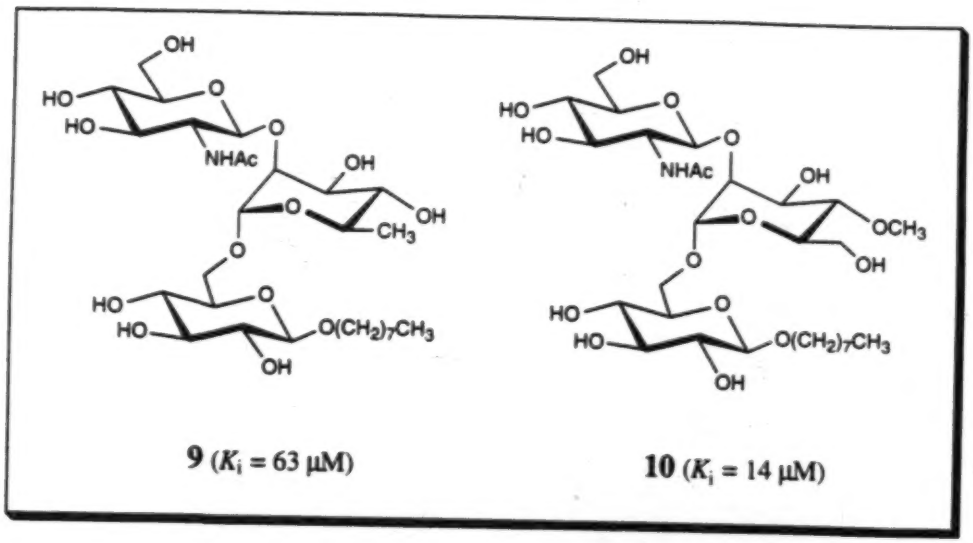

Fig. 16. Two competitive trisaccharide inhibitors for GlcNAcT-V

To investigate the involvement of the three hydroxyl groups of the β-GlcNAc residue of **5** in the recognition by GlcNAcT-V, trisaccharides where this residue was modified at OH-3, OH-4 and OH-6 groups were synthesized and enzymatically evaluated. OH-4 was replaced by H, OMe, F, NH_2, NHAc and epimerized. None of the resulting analogs were recognized by GlcNAcT-V [95]. Compounds where OH-3 of the GlcNAc residue was replaced with either NH_2 or NHAc retained less than 1% of the activity of the parent synthetic acceptor **5** [96]. Trisaccharides where OH-6 of the GlcNAc residue was replaced by NH_2 or NHAc showed no acceptor activity [96]. Furthermore, none of the analogs modified at the 3-, 4- and 6- positions of the GlcNAc residue showed any inhibitory activity. Those results indicated that OH-3, OH-4 and OH-6 of the GlcNAc residue are essential for recognition by the enzyme (Fig. 17).

The role of the β-Glc residue in the recognition by GlcNAcT-V has also been studied. Deoxygenation of OH-4 of the β-D-Glc residue in **5** had little effect on the activity, while the corresponding 4-*O*-methyl derivative was twice as active [88]. Analogs of **5**

where the Glc-residue was tri-*O*-methylated or tri-*O*-benzylated possessed K_m values near to the K_m of **5** (Fig. 17) [97]. The disaccharide which lacks the Glc residue of acceptor **5** was found to be a very poor substrate. The pseudo-trisaccharide where the β-Glc residue of **5** was substituted by cyclohexylmethyl residue retained only 25% of the activity of **5** [88]. Those results indicated that the Glc residue presents an important recognition element for GlcNAc T-V, but none of the free hydroxy groups are required for the binding.

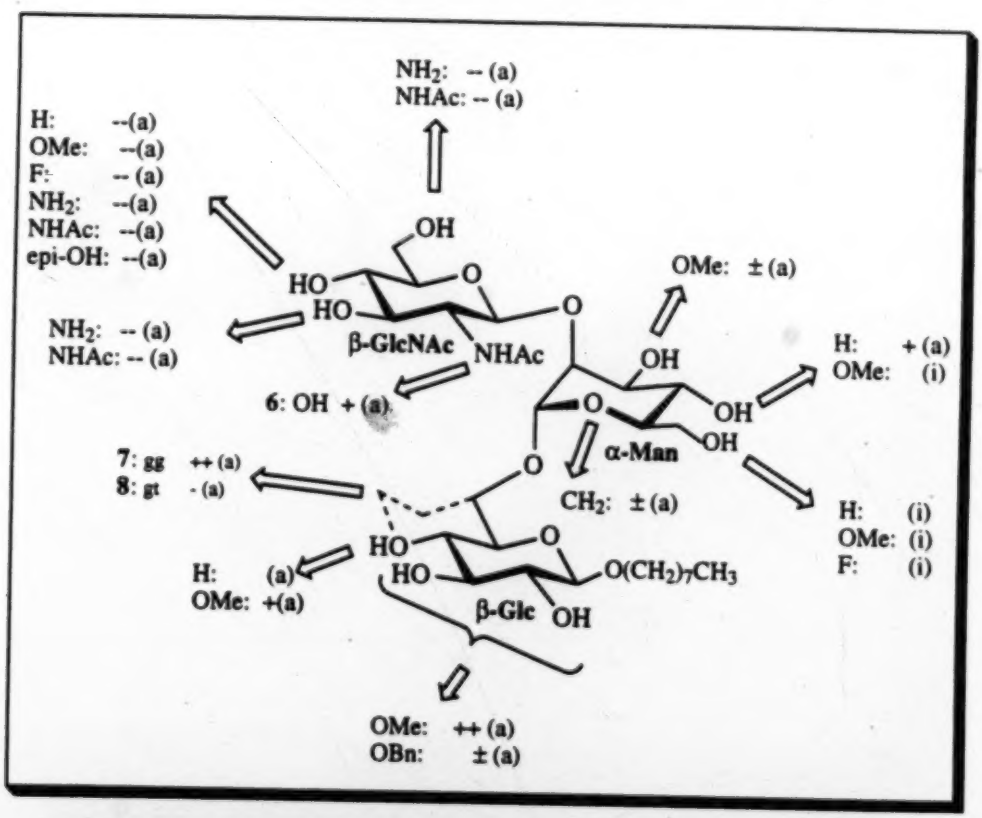

Fig. 17: Summary of the substrate specificity of GlcNAcT-V. The letters (a) and (i) indicate acceptor and inhibitor, respectively. Acceptor activities are relative to 5. The efficiency (V_{max}/K_m) for each compound is relative to 5 taken as 100%. ±, same range (70-130%); +, slightly better (130-200%); ++, better (>200%); -, slightly worse (10-70%); --, very bad (<10%).

S. Ogawa's group, in collaboration with the Palcic laboratory, recently reported the synthesis of a carbocyclic analog of **5**, where the ring oxygen atom of the central mannose residue was replaced by a methylene group. This compound was found to be a fully active acceptor for GlcNAcT-V [98]. This work demonstrated that the ring oxygen of the central mannose residue in **5** is not involved in specific recognition by GlcNAcT-V.

B. Work of other laboratories

In 1989, Matta's group reported the synthesis of three trisaccharides, β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Man*p*-O-$C_6H_4NO_2$, β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Glc*p*-O-$C_6H_4NO_2$, β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Glc*p*-O-Allyl and one tetrasaccharide β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Glc*p*-(1→4)-β-D-Glc*p*-O-Allyl as potential substrates for GlcNAcT-V [99]. These three trisaccharides are similar to acceptors **3** and **5**, but have different aglycons. In 1990, the same group reported the synthesis of the trisacccharide β-D-Glc*p*NAc-(1→2)-4-*O*-methyl-α-D-Man*p*-(1→6)-β-D-Glc*p*-*O*-$C_6H_4NO_2$ where OH-4 of the central mannose residue was methylated as a potential acceptor-substrate [100]. A derivative (**10**) of this compound, which has the octyl group replacing the nitrophenyl aglycon, was later shown by Khan *et al* in fact to inhibit the enzyme [94].

Khan and Matta also synthesized two trisaccharide analogs of β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Glc*p*-O-$C_6H_4NO_2$, having 6-methoxy and 4,6-dimethoxy substituents on the central mannose residues respectively, as potential inhibitors of GlcNAcT-V [101].

The tetrasaccharides β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Glc*p*-(1→4)-β-D-Glc*p*NAc [102] and β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Man*p*-(1→4)-β-D-

Glc*p*NAc [103] were synthesized in Matta's group as potential acceptor-substrates for GlcNAcT-V. Although the latter is a larger partial structure of natural heptasaccharide substrate 4, it was very poorly recognized by the enzyme in a preliminary enzyme evaluation.

H. Paulsen's group synthesized the pentasaccharide β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→3)-[β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)]-β-D-Man*p*-octyl and a series of modified derivatives of this pentaccharide. OH-4 of the β-Man residue, OH-3 or OH-4 of the α-(1→3)-linked Man residue or OH-6 of the α-(1→6)-linked Man residue were deoxygenated. The resultant pentasaccharides were used in substrate specificity studies of GlcNAcT-V and other GlcNAc transferases (III, IV, VI) [104-106].

In summary, the following compounds which were chemically synthesized in Matta's or Paulsen's laboratories have been enzymatically evaluated as substrates and inhibitors of GlcNAcT-V [106] (all = allyl, oct = octyl, pnp = *p*-nitrophenyl):

β-GlcNAc(1→2)-α-Man(1→6)-β-Glc-*O*-pnp,

β-GlcNAc(1→2)-α-Man(1→6)-β-Glc-*O*-all,

β-GlcNAc(1→2)-α-Man(1→6)-β-Glc(1→4)-β-Glc-*O*-all [99];

β-GlcNAc(1→2)-α-Man(1→6)-β-Man-*O*-Me,

β-GlcNAc(1→2)-α-Man-*O*-Me,

β-GlcNAc(1→4)-[β-GlcNAc(1→2)]-α-Man-*O*-Me,

β-GlcNAc(1→6)-α-Man-*O*-Me [99];

β-GlcNAc(1→2)-α-Man(1→6)-β-Glc(1→4)-β-GlcNAc,

β-GlcNAc(1→2)-6-*O*-Me-α-Man(1→6)-β-Glc-*O*-pnp,

β-GlcNAc(1→2)-4,6-di-*O*-Me-α-Man(1→6)-β-Glc-*O*-pnp [102];

β-GlcNAc(1→2)-4-*O*-Me-α-Man(1→6)-β-Glc-*O*-pnp [100];

β-GlcNAc(1→2)-6-*O*-Me-α-Man-*O*-Me,

β-GlcNAc(1→2)-4,6-di-*O*-Me-α-Man-*O*-Me [101];

β-GlcNAc(1→2)-α-Man(1→6)-β-Glc-*O*-oct,

β-GlcNAc(1→2)-α-Man(1→6)-[β-GlcNAc(1→2)-α-Man(1→3)]-β-Man-*O*-oct,

β-GlcNAc(1→2)-α-Man(1→6)-[β-GlcNAc(1→2)-3-deoxy-α-Man(1→3)]-β-Man-*O*-oct,

β-GlcNAc(1→2)-α-Man(1→6)-[β-GlcNAc(1→2)-4-deoxy-α-Man(1→3)]-β-Man-*O*-oct,

β-GlcNAc(1→2)-α-Man(1→6)-[β-GlcNAc(1→2)-α-Man(1→3)]-4-*O*-Me-β-Man-*O*-oct,

β-GlcNAc(1→2)-α-Man(1→6)-[β-GlcNAc(1→2)-α-Man(1→3)]-4-deoxy-β-Man-*O*-oct,

β-GlcNAc(1→2)-α-Man(1→6)-[β-GlcNAc(1→2)-4-deoxy-α-Man(1→3)]-4-*O*-Me-β-Man-*O*-oct [104];

3-*O*-pivaloyl-β-GlcNAc(1→2)-α-Man(1→6)-β-Glc-*O*-pnp.

Kinetic evaluations of these compounds indicated that the nature of the aglyconic group had a strong influence of the effectiveness of the substrate, with the octyl compounds giving the highest activities. Compounds with a biantennary structure were significantly better substrates than those with only the linear β-GlcNAc-(1→2)-α-Man-(1→6)-β-Man/β-Glc structure. The K_m values of the biantennary derivatives ranged from 35 to 180 μM and enzyme activities were relatively high when compared to the linear substrate. Kinetic analysis showed that OH-3 of the α-Man(1→3) residue and OH-4 of the β-Man residue of the biantennary structures are not essential for catalysis but influence substrate binding. *O*-Methylation of OH-4 on the internal β-Man residue increased activity but omission of this hydroxyl increased the K_m 3-fold. Omission of the 4-hydroxy of the α-Man(1→3) residue of the biantennary substrate had little effect on activity whereas the 3-deoxy analog was less active. Activities towards linear tetrasaccharide substrates were within the same range as for trisaccharides but the disaccharide β-GlcNAc(1→6)-α-Man-*O*-Me was significantly less active. Inhibition studies demonstrated that β-GlcNAc(1→2)-4-*O*-Me-α-Man(1→6)-βGlc-*O*-pnp, β-GlcNAc(1→2)-6-*O*-Me-α-Man(1→6)-β-Glc-*O*-

pnp and β-GlcNAc(1→2)-4,6-di-*O*-Me-α-Man(1→6)-β-Glc-*O*-pnp [102] were inhibitors of GlcNAcT-V, but K_i values were not determined [106].

The specificity of GlcNAcT-V towards the biantennary substrate β-GlcNAc(1→2)-α-Man(1→6)-[β-GlcNAc(1→2)-α-Man(1→3)]-β-Man-O-R is summarized in Fig. 18 according to reference[106].

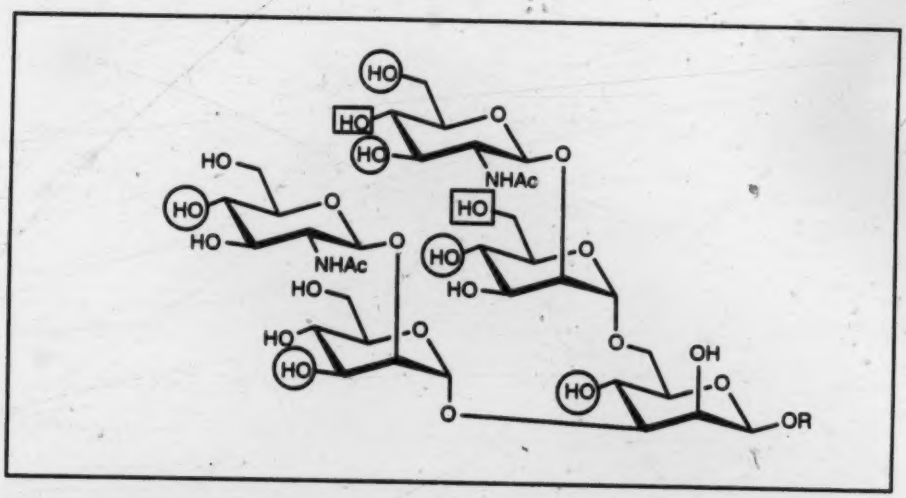

Fig. 18. GlcNAcT-V specificity towards a biantennary pentasaccharide substrate. The hydroxyls found to be essential for activity are surrounded by squares. Hydroxyls that have an influence but are not essential for activity are surrounded by circles. (According to ref. [106]).

1.10 Scope of the Thesis Research

This thesis research has the objective of providing active-site-directed specific inhibitors for GlcNAcT-V. The work involves the design and synthesis of new trisaccharide analogs as inhibitors of GlcNAcT-V, the synthesis of thioglycosides as acceptors for GlcNAcT-V, and finally the synthesis and conformational analysis of diastereospecifically deuterated trisaccharide acceptors.

CHAPTER 2

New Synthetic Trisaccharide Inhibitors for *N*-Acetylglucosaminyltransferase-V[a,b]

2.1 Introduction

This chapter describes the design, synthesis and enzymatic testing of trisaccharide analogs **11a-11k**, **12a-12j** and **13** (Fig. 19) as inhibitors for GlcNAcT-V.

The 4'-*O*-methyl derivative (**10**) (Fig. 16) of **5** was previously found to be a good competitive inhibitor of GlcNAcT-V with K_i = 14 μM [94]. It was suggested that the 4-*O*-methyl group on the central Man residue sterically prevented the formation of product even though both donor and acceptor substrates were bound by the enzyme, since the 4'-deoxy derivative of **5** is a good acceptor for GlcNAcT-V [94]. Therefore, we decided to investigate whether trisaccharides of the general structure **11**, substituted with a free amino group (NH_2) at the position to which GlcNAcT-V transfers, could be used to probe the characteristics of the protein structure near the active site of the enzyme by evaluating them as potential inhibitors. The presence of the 4'-*O*-methyl group would secure that transfer to position 6 of the central Man residue could not occur in any of the analogs, which would potentially complicate interpretation of the results, while the 6'-amino group would allow the facile preparation of derivatives from a single precursor primary amine by taking advantage of the distinct reactivity of amino group. Analogs of **11a** would be prepared by

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[a] The enzyme inhibition experiments reported in this chapter were performed by Dr. Hong Li and Ms. Catharine A. Compston in the group of Prof. Monica M. Palcic.

[b] Part of this work has been published [108].

acylation and alkylation with hydrophobic, hydrophilic, aromatic and charged residues. Potential protein derivatizing groups (such as iodoacyl, Michael acceptors etc.) at this position attached to this group were also envisioned.

The 6'-deoxy-derivative (**9**) of trisaccharide **5**, where the reactive OH-6' had been removed, was the first reported inhibitor specific for a glycosyltransferase and had a K_i value in the range 30-70 μM depending on the source of the enzyme [93]. It was also found that GlcNAcT-V tolerates substitutions at *O*-4 of the central Man residue, since 4'-deoxy analog of **5** was found to be a good acceptor for GlcNAcT-V, while the 4'-*O*Me analog (**10**) of **5** was a competitive inhibitor [94] as noted above. In order to examine the tolerance of GlcNAcT-V to substitution at C-4 of the α-Man residue, and to produce potentially more potent inhibitors, we designed trisaccharides of general structure **12** as potential inhibitors. In the structure of **12**, C-6 of the α-Man residue has been deoxygenated so that the analogs of **12** can only potentially act as inhibitors, not acceptors, to simplify their evaluation as ligands for GlcNAcT-V. In order to simplify the preparation of the required series of active-site probes, an amino group was installed at C-4 of the α-Man residue based on the same strategy mentioned above. The distinct chemical reactivity of this amino group permitted the facile derivation of a single deprotected trisaccharide (**12a**, *i.e.* **12** with R = H) to yield the required target analogs.

In this chapter, trisaccharide **13**, differing from **12** in that the terminal GlcNAc moiety was replaced by a β-Glc residue, was also synthesized and evaluated as an inhibitor for GlcNAcT-V. Potential photo-affinity labels based on trisaccharides **11a** and **12a** were also successfully synthesized. Such photogenerated reagents should be useful in investigating the interaction between the acceptor and the enzyme [109-112].

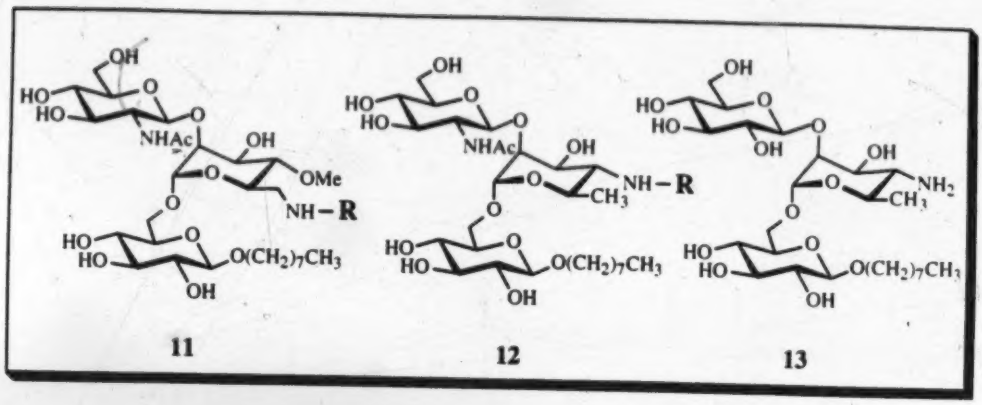


Fig. 19. Designed trisaccharide inhibitors for GlcNAcT-V

2.2 Chemical Synthesis of Trisaccharide 11a

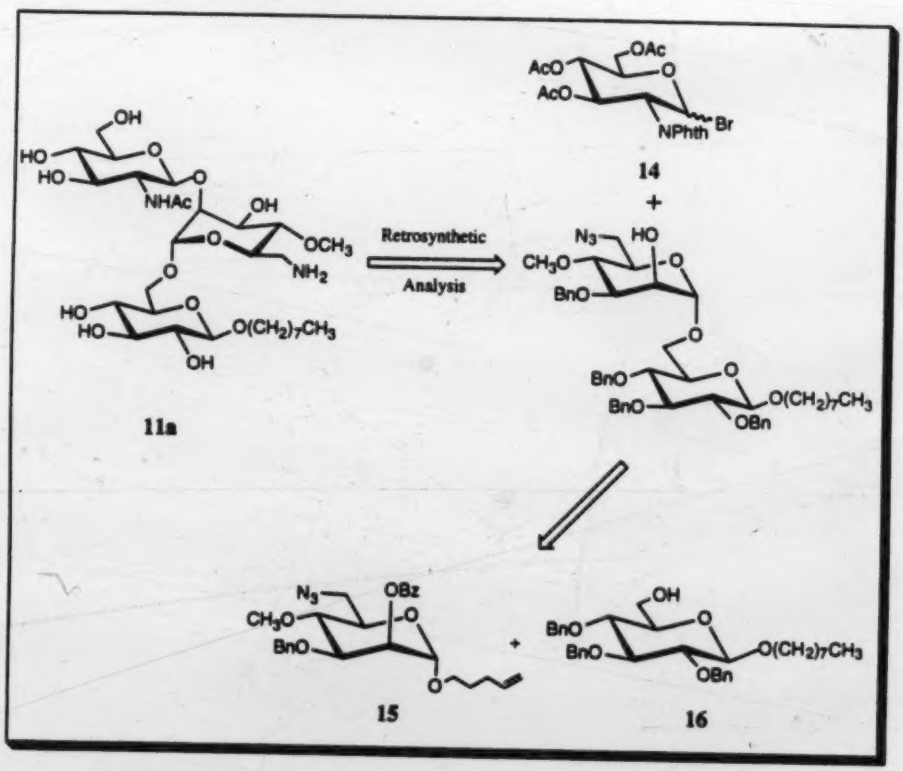


Fig. 20. Strategy for the synthesis of trisaccharide **11a**

Retrosynthetic analysis suggested that the synthesis of the target molecule could be achieved by the sequential coupling of building blocks **14-16** (Fig. 20). The bottom building block **16** was prepared from D-glucose as previously described [88, 113] (Fig. 21).

For the synthesis of the key building block **15**, D-mannose was used as the starting material (Fig. 22). Direct glycosylation of 4-penten-1-ol with D-mannose produced 4-pentenyl α-D-mannopyranoside (**22**) [114]. Treatment of **22** with 4-methoxybenzaldehyde dimethyl acetal and pyridinium *p*-toluenesulfonate (PPTS) in DMF at 80 °C led to selective benzylidenation [115] to give 4-pentenyl 4,6-*O*-(4-methoxybenzylidene)-α-D-mannopyranoside (**23**) in 46% yield. Compound **23** was then selectively benzylated, *via* its 2,3-*O*-dibutylstannylidene derivative [116], to provide the 3-*O*-benzyl derivative (**24**) in 86% yield. Benzoylation of OH-2 followed by regioselective reductive ring-opening of the 4-methoxybenzylidene acetal in **25** with sodium cyanoborohydride-trifluoroacetic acid in DMF produced the 6-*O*-(4-methoxybenzyl) ether (**26**) in 85% yield [117]. Methylation of OH-4 in **26** in the presence of the benzoyl ester group was achieved using methyl iodide and sodium hydride in DMF at -15 °C followed by immediate neutralization with acetic acid. The use of cerium (IV) ammonium nitrate (CAN) for the selective deprotection of the *p*-methoxybenzyl group was attempted, but was not successful. Treatment of **27** with DDQ in dichloromethane saturated with water [118] afforded **28** with OH-6 in 87% yield. Tosylation of OH-6 in **28**, followed by displacement with sodium azide, gave the key building block **15**.

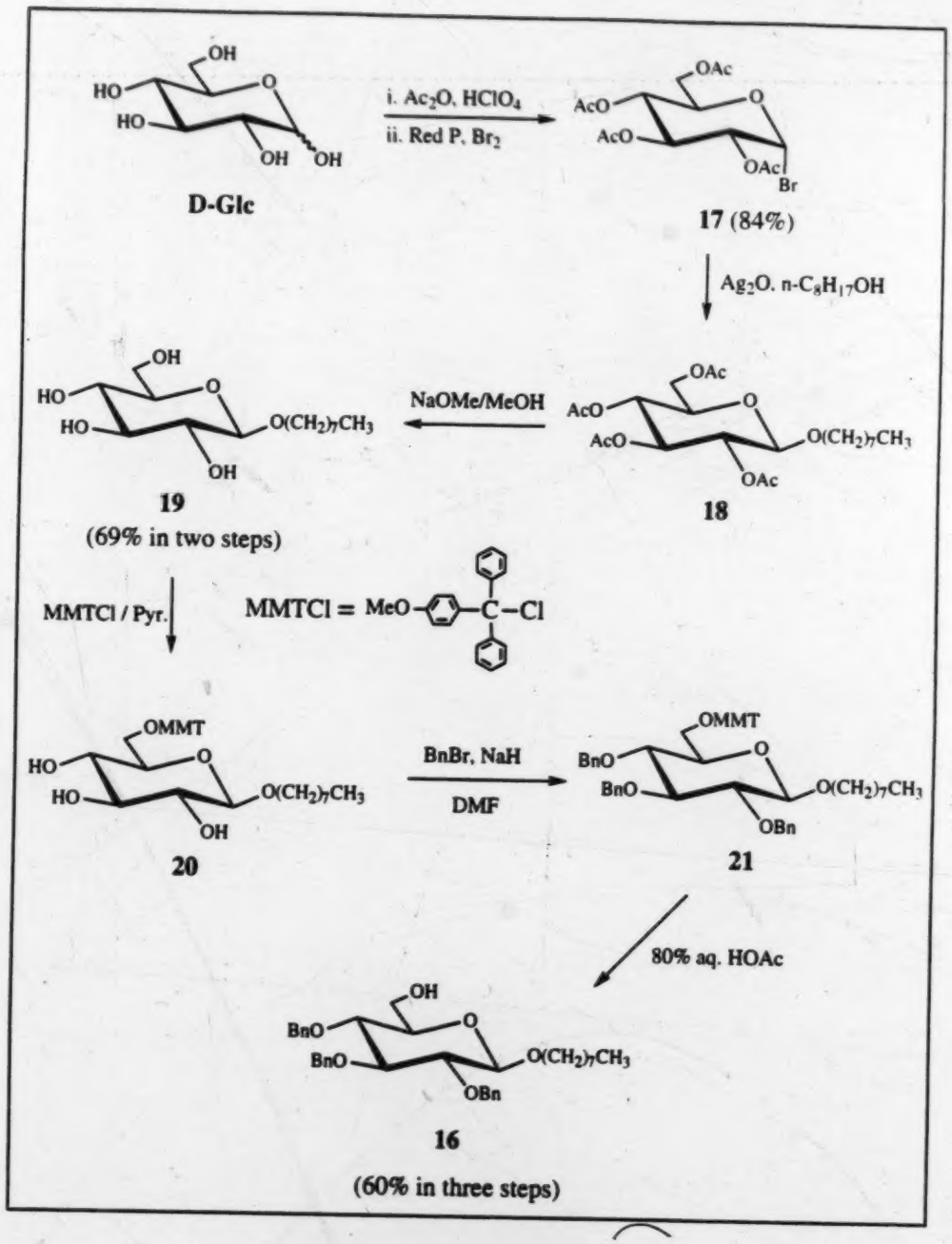


Fig. 21. Synthesis of building block **16**

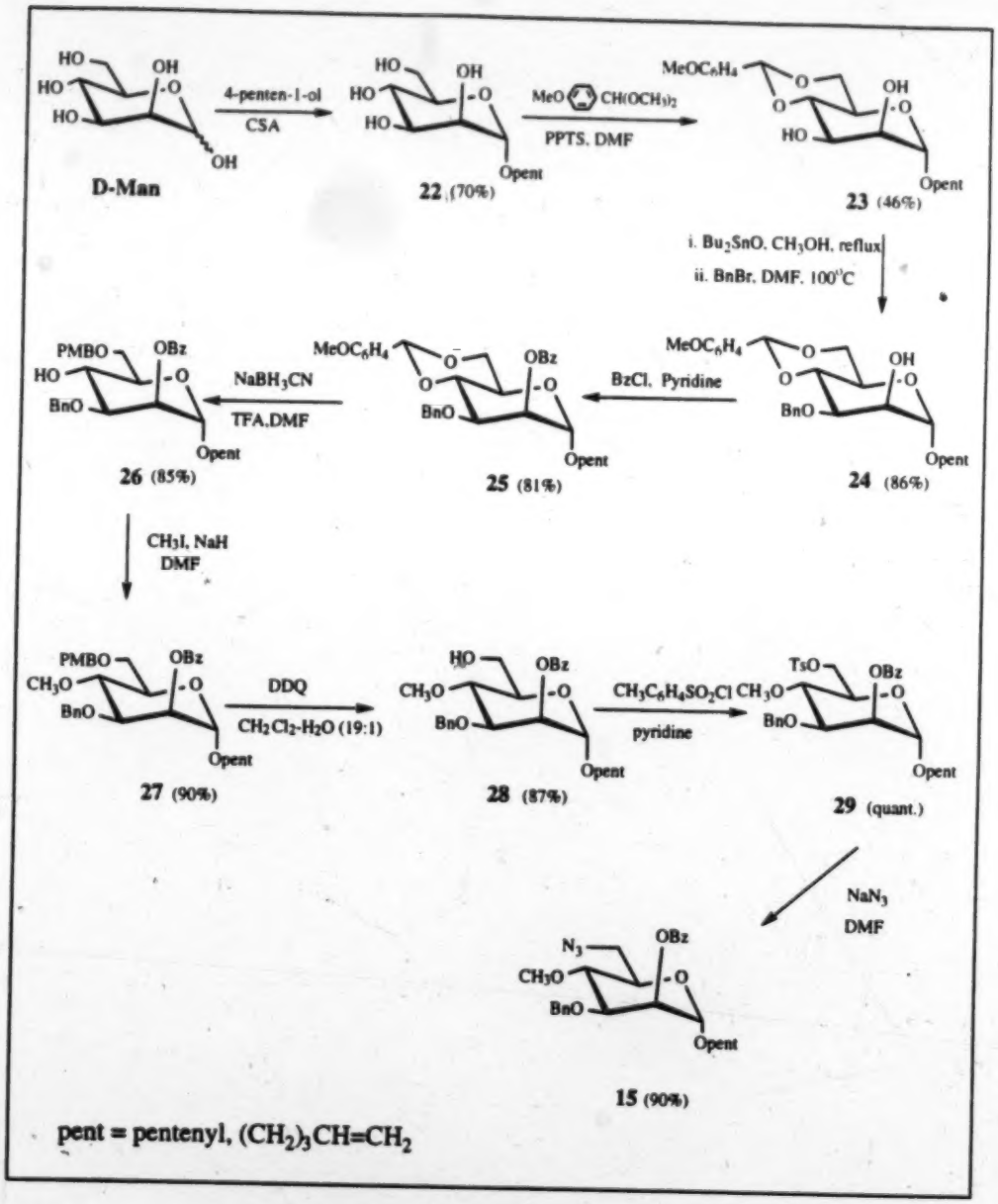


Fig. 22. Synthesis of building block **15**

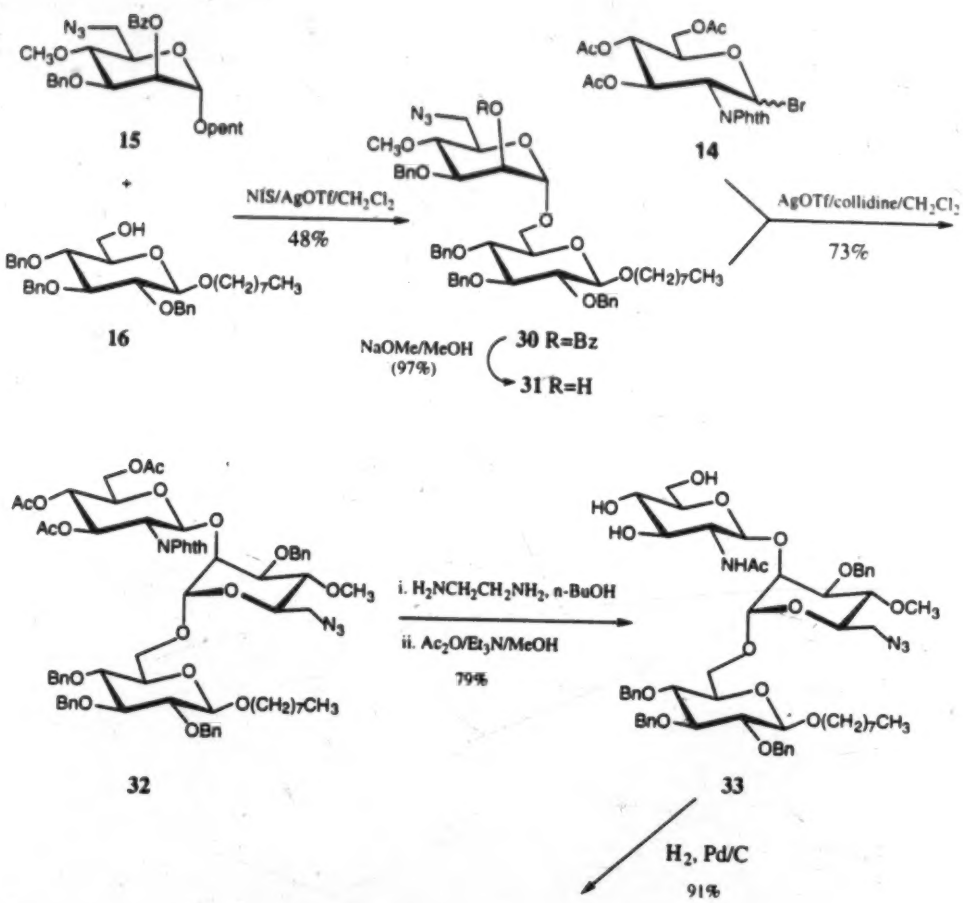


Fig. 23. Assembly of trisaccharide **11a**

The coupling of **16** [88] and **15** using *N*-iodosuccinimide (NIS) and silver trifluoromethanesulfonate (AgOTf) as promoters [114] furnished the α-linked disaccharide **30** in 48% yield. The anomeric configuration was confirmed by its ^{13}C-NMR spectrum which showed the anomeric carbons resonating at δ 103.6 ($J_{C\text{-}1,H\text{-}1}$ = 161.5 Hz) and 97.9 ($J_{C\text{-}1',H\text{-}1'}$ = 170.9 Hz) respectively. One-bond C-H coupling constants of these magnitudes require the presence of the β and α glycosidic linkages, respectively [119]. Deacetylation of **30** with methanolic sodium methoxide gave **31** with the OH-group at C-2 free as required for further coupling. Condensation of **31** and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide (**14**) [120] using AgOTf as promoter provided the trisaccharide **32** in 72% yield. Removal of the *N*-phthalimido and *O*-acetyl protecting groups was achieved using 1,2-diaminoethane in butanol [95]. *N*-Acetylation of the resultant free amine with acetic anhydride in dry methanol gave **33** in 79% overall yield from **32**. Finally, hydrogenolytic cleavage of the benzyl protecting groups and the reduction of the azido group of **33** using 5% palladium-on-charcoal as the catalyst, in 95% ethanol (aldehyde free), furnished the target trisaccharide **11a** in 91% yield (Fig. 23).

2.3 Chemical Synthesis of Trisaccharide Analogs (11b-11k)

The required analogs (**11b-11k**) were prepared conventionally using the reagents indicated in Table I. Briefly, the *N*-acetylated analog (**11b**) was prepared by treatment of **11a** with acetic anhydride in methanol. The *N*-benzoylated derivative (**11c**) was obtained by treatment of **11a** with benzoyl chloride in pyridine to give the perbenzoylated compound, followed by *O*-debenzoylation with NaOMe/MeOH. Compound **11d**, with a negative charge at physiological pH, was produced by treatment of **11a** with succinic anhydride in methanol. The trisaccharide bearing a potentially reactive *N*-iodoacyl group (**11e**) was synthesized by reaction of **11a** with iodoacetic anhydride in methanol. The trisaccharide containing a potential Michael acceptor residue was prepared from **11a** by

acylation with acryloyl chloride in DMF, giving **11f**. An analog bearing an aromatic residue (**11g**) was obtained by reaction of **11e** with thiophenol. An analog with a positive charge (**11h**) was prepared by reaction of **11e** with *N,N*-dimethylethylamine. A derivative bearing a nitrophenyl residue (**11i**) was obtained by reaction of **11a** with 2,4-dinitrofluorobenzene. The analog containing a dansyl residue (**11j**) was obtained by treatment of **11a** with dansyl chloride in DMF. The potential photo-affinity label **11k** was prepared by reaction of **11a** with *p*-nitrophenyl 2-diazo-3,3,3-trifluoropropanoate in DMF [111, 112] (Fig. 24). All of the analogs (**11a-11k**) were characterized by ^{1}H NMR and FAB MS.

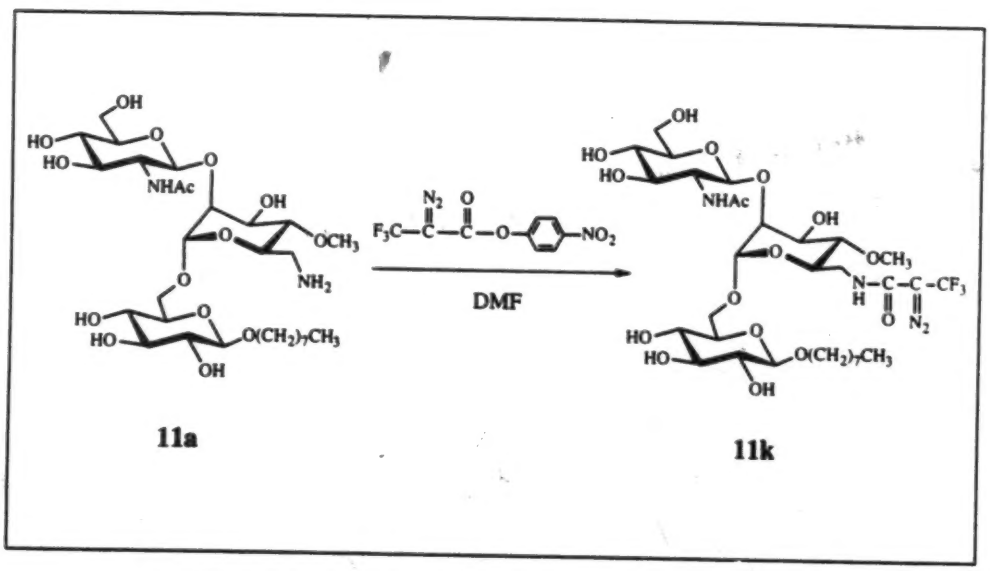


Fig. 24. Synthesis of the potential photo-affinity label **11k**

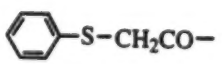
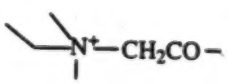
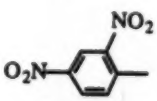
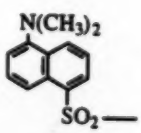
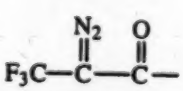
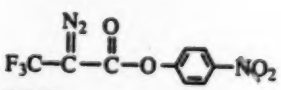
2.4 Enzymatic Evaluation of 11a-11k as Inhibitors of GlcNAcT-V

Compounds **11a-11k** were tested as inhibitors for GlcNAcT-V which was partially purified from hamster kidney following published procedures [95]. The activity of the

synthetic trisaccharide derivatives were determined by a radioactive assay technique as previously described [88, 94]. The results of the evaluation of trisaccharides **11a-11j** as inhibitors are presented in Table I. Compound **11a** and all of the analogs were shown to be competitive substrate inhibitors of GlcNAcT-V [108] (data not shown). It appears, therefore, that GlcNAcT-V can tolerate the replacement of the active 6'-hydroxyl group of the central mannose residue with a potentially active amino group. The enzyme can further tolerate the diverse modifications of the 6'-amino group that are obtained by adding various groups such as acetyl, iodoacyl, aromatic and Michael acceptors, etc. In general, acyl groups at this position increase inhibition. The presence of a potential alkylation agent (iodoacyl) at this position caused stronger inhibition which was not time-dependent, indicating that the enzyme was not alkylated. Groups at this position with steric bulk decrease inhibition and there may be decrease associated with a positive charge although this has not been fully tested.

The binding of photo-affinity label derivative (**11k**) with recombinant GlcNAcT-V is being examined.

Table I. Evaluation of trisaccharides **11a-11k** as Inhibitors of GlcNAcT-V[a]

Compound	R —	Reagents Used for Amino Derivatization	K_i (μM)
11a	H		297
11b	CH_3CO-	Ac_2O / MeOH	88
11c	C_6H_5CO-	i. C_6H_5COCl / Py ii. NaOMe / MeOH	36
11d		i. succinic anhydride / MeOH ii. $NaHCO_3$ aq.	31
11e	ICH_2CO-	$(ICH_2CO)_2O$ / MeOH	21
11f	$CH_2=CHCO-$	$CH_2=CHCOCl$ / DMF / $NaHCO_3$	45
11g		i. $(ICH_2CO)_2O$ / MeOH ii. C_6H_5SH / $NaHCO_3$ aq.	29
11h		i. $(ICH_2CO)_2O$ / MeOH ii. $(CH_3)_2NC_2H_5$ / MeOH	175
11i		2, 4 - Dinitrofluorobenzene / Phosphate buffer	32
11j		Dansyl Chloride / DMF / $NaHCO_3$ aq.	145
11k		 DMF	18

[a] Experimental errors ±5%

2.5 Chemical Synthesis of 12a and 13

For the synthesis of **12a**, retrosynthetic analysis suggested that **14**, **16** and **34** could be used as monosaccharide precursors (Fig. 25).

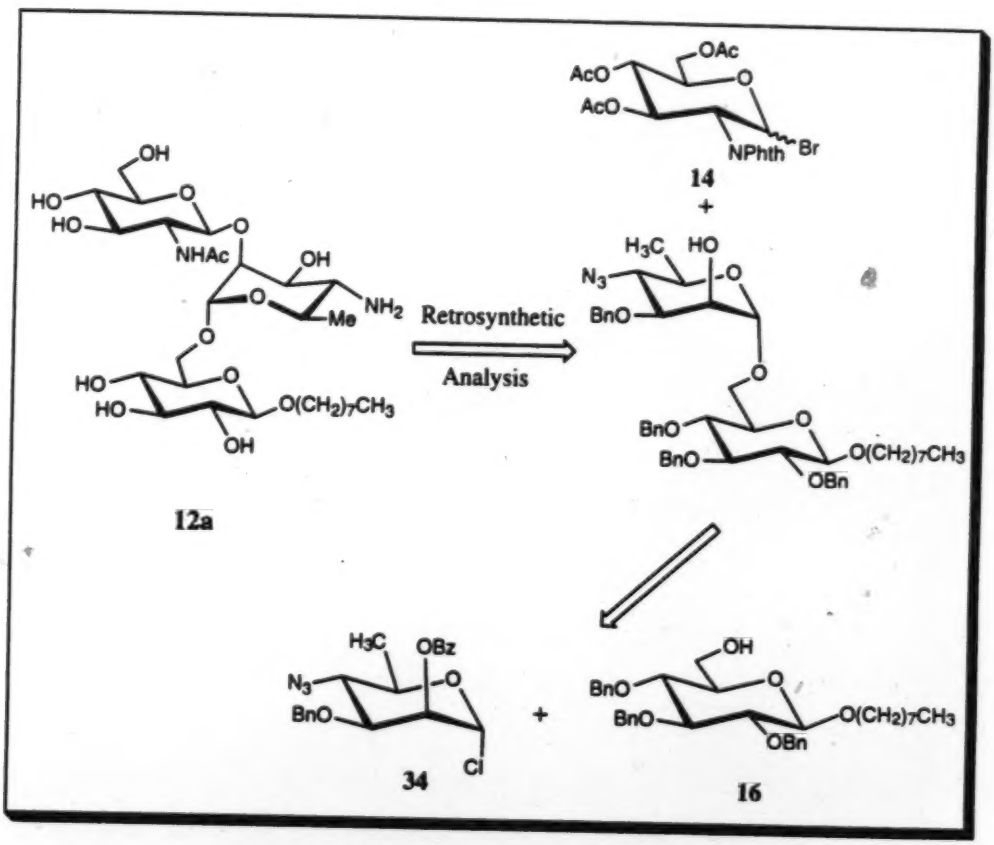


Fig. 25. Retrosynthetic analysis of trisaccharide **12a**

Compounds **14** and **16** were synthesized as described above. The key central building block **34** was synthesized as described by Bundle [121a] and Ganem [121b] with slight modification (Fig. 26). The modification was the regioselective benzylation of **40** at the 3 position.

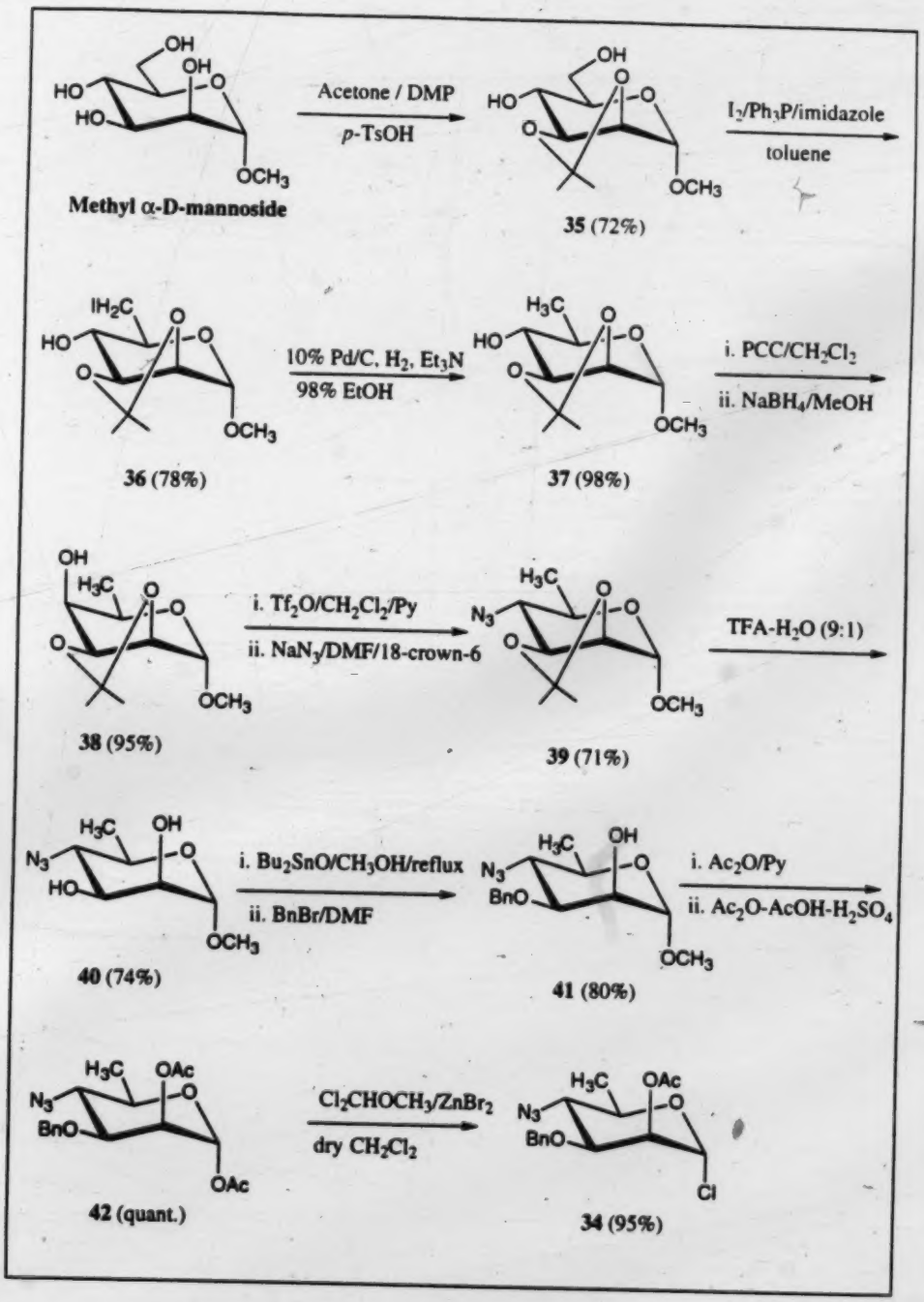


Fig. 26. Synthesis of building block **34**

Compound **40** was selectively benzylated, *via* its 2,3-*O*-dibutylstannylidene derivative [116], to provide the 3-*O*-benzyl derivative (**41**) in 80% yield.

The coupling of the key building block **34** [121] and alcohol **16** [88] using silver trifluoromethanesulfonate (AgOTf) as promoter gave the α-linked disaccharide **43** in 68% yield. The anomeric configuration was confirmed from its ^{1}H-coupled ^{13}C NMR spectrum which revealed a doublet each for C-1 and C-1' at δ 103.54 (C-1, $J_{C\text{-}1,H\text{-}1}$ = 156.9 Hz) and 97.73 (C-1', $J_{C'\text{-}1,H\text{-}1'}$ = 170.9 Hz) respectively [119].

Zemplén deacetylation of **43** yielded **44** with OH-2' free for further reaction. Glycosylation of alcohol **44** with glycosyl bromide **14** [120] was carried out in the presence of AgOTf and collidine to give trisaccharide **45** in 77% yield. The removal of the *N*-phthalimido and acetyl groups in **45**, and subsequent *N*-acetyiation of the free amine, were achieved by the use of 1,2-diaminoethane / butanol [95] followed by treatment with acetic anhydride in dry methanol. Compound **46** was obtained in 88% overall yield.

The removal of benzyl groups and concomitant reduction of the azido group in **46** were attempted by using hydrogenation (H_2, Pd/C) under numerous conditions (different solvents, 1 eq. HCl or acetic acid), but the hydrogenations were found to be sluggish and the yields were very low. Finally, the debenzylation and concomitant reduction of the azido group were achieved by Birch reduction (Na / liq. NH_3) [122] to give the amino target trisaccharide **12a** in 81% yield (Fig. 27).

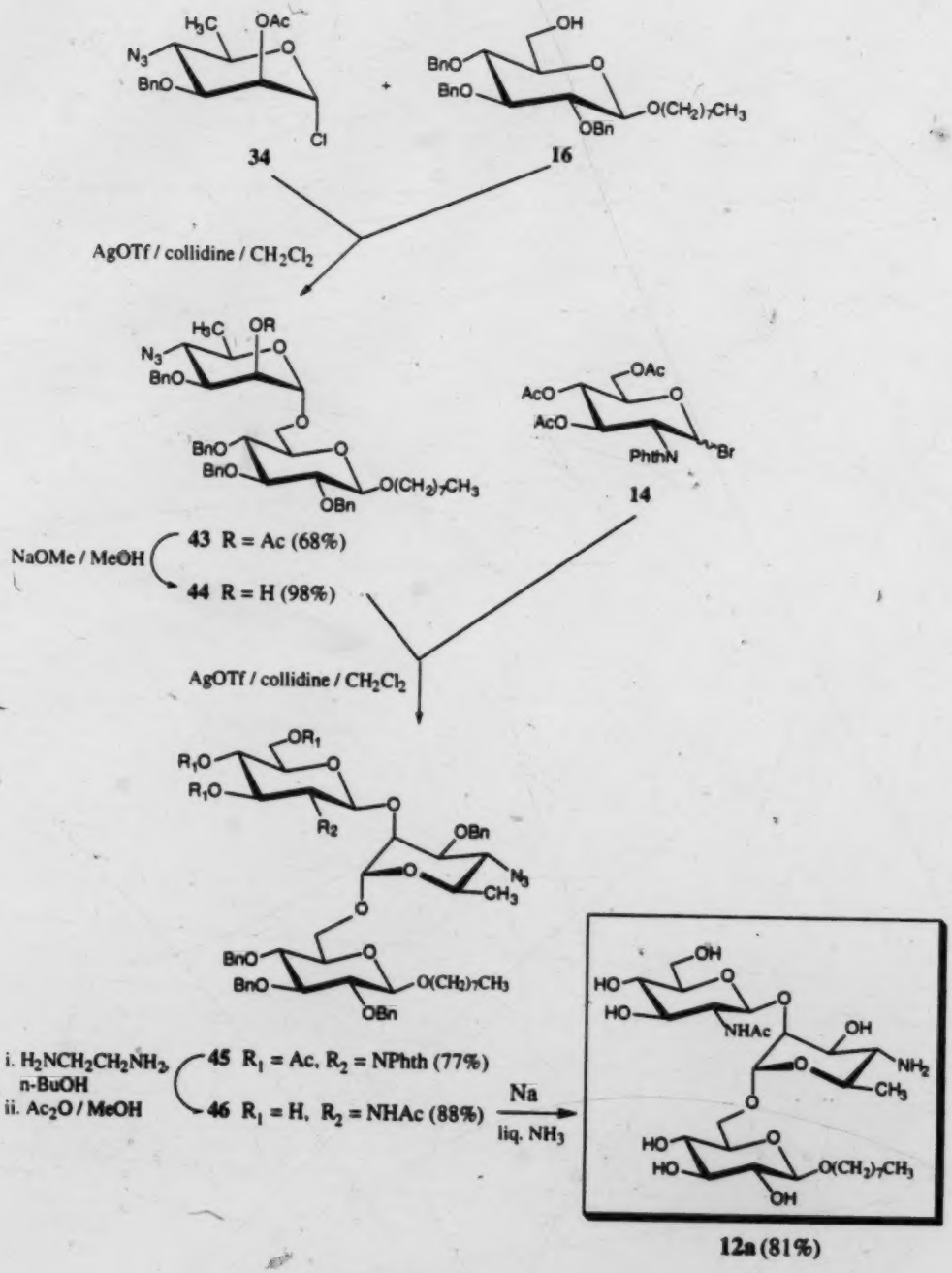


Fig. 27. Synthesis of trisaccharide **12a**

To prepare trisaccharide **13**, 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucosyl bromide were explored as donors, using $Hg(CN)_2$ / $HgBr_2$ or AgOTf as promoters to couple with alcohol **44**, but without success. The coupling of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**47**) [123] and alcohol **44** using AgOTf as promoter afforded trisaccharide **48** in 31% yield. The glucopyranosyl bromide **47** was prepared directly from 3,4,6-tri-*O*-benzyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose [124] by treatment with acetyl bromide in the presence of tetraethylammonium bromide [123]. Zemplén deacetylation of **48** followed by Birch reduction provided the target trisaccharide **13** in 47% yield.

2.6 Chemical Synthesis of Trisaccharide Analogs (12b-12j)

The trisaccharide analogs (**12b-12h**) were prepared from **12a** using the reagents indicated in Table II for selective *N*-derivatization [108]. All of the analogs were characterized by ^1H NMR spectroscopy and FAB MS.

Two compounds bearing potential photo-affinity labels (**12i, 12j**) were also synthesized. Attempts at the preparation of **12i** by treatment of **12a** with *p*-nitrophenyl 2-diazo-3,3,3-trifluoropropanoate [111, 112] were not successful. Compound **12i** was obtained by reaction of **12a** with 2-diazo-3,3,3-trifluoropropanoyl chloride in a mixture of DMF and aq. $NaHCO_3$. Compound **12j** was produced by treatment of **12a** with *N*-hydroxysulfosuccinimidyl 4-azidobenzoate in 0.1 N aq. $NaHCO_3$ (Fig. 29).

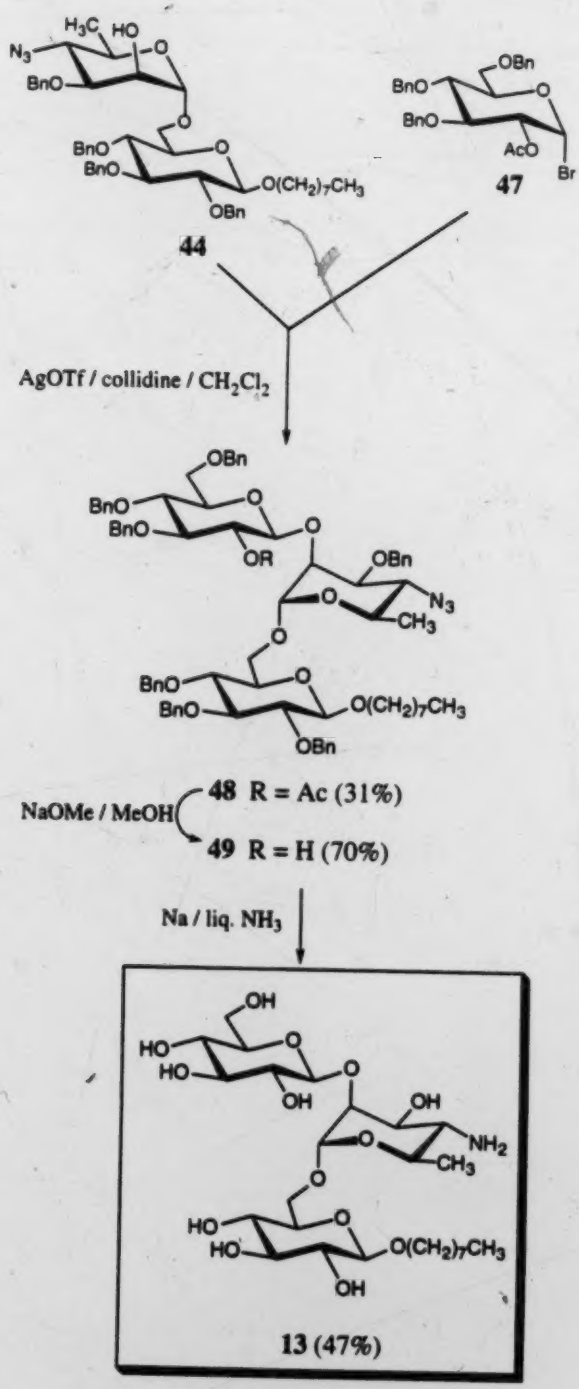


Fig. 28. Synthesis of trisaccharide **13**

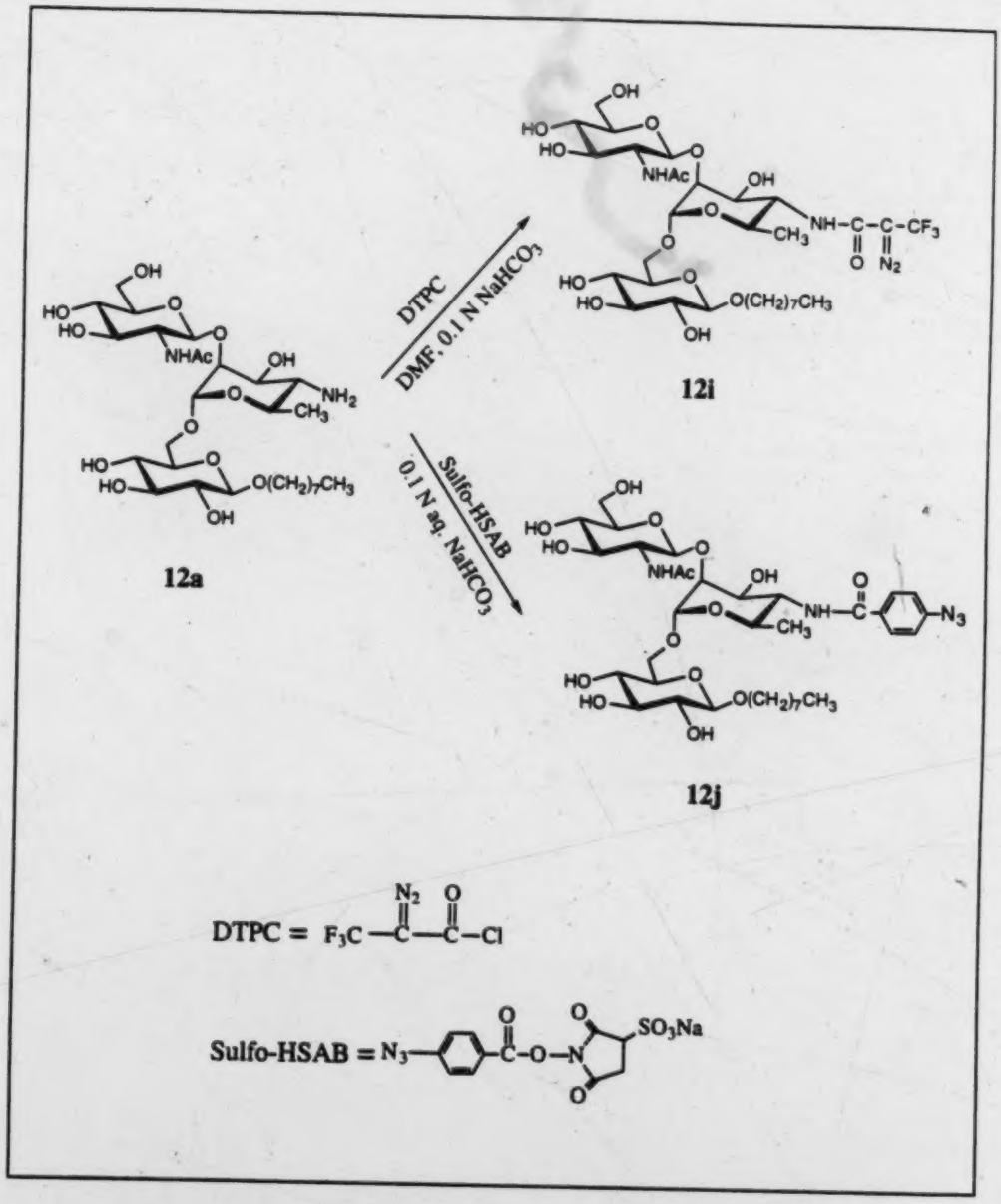


Fig. 29. Synthesis of the potential photo-affinity labels **12i** and **12j**

2.7 Enzymatic Evaluation of 12a-12j and 13 as Inhibitors of GlcNAcT-V

Compounds **12a-12j** and trisaccharide **13** were evaluated as inhibitors of GlcNAcT-V, both partially purified from hamster kidney [94] and cloned (from rat kidney) [84]. The activity of the synthetic trisaccharide derivatives were determined using a radioactive assay technique (the "Sep-Pak" assay) as previously described [90, 94]. Results of the evaluation of trisaccharides **12a-12j** and trisaccharide **13** as inhibitors are presented in Table II. All of the analogs were shown to be competitive inhibitors of GlcNAcT-V. The results confirm that the removal of the reactive 6'-OH group produces competitive inhibitors of GlcNAcT-V [93]. The results demonstrate that GlcNAcT-V tolerates very diverse modifications at C-4' without loss of recognition. Replacement of a hydrogen by acyl or aryl groups at the NH_2-4' group increases the inhibitor activity, supporting the conclusion that increasing the bulk of the groups on the 4'-position sterically prevented the formation of product even though the potential substrates were bound by the enzyme [94]. This size effect clearly has an upper bound since the aryl group causes the most effective inhibition. Trisaccharide **13** was also shown to be a competitive inhibitor of GlcNAcT-V. This result further demonstrates that the enzyme tolerates the replacement of the terminal GlcNAc moiety by D-glucose [89].

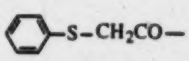
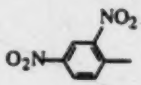
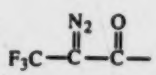
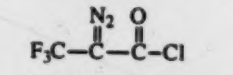
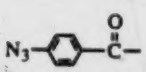
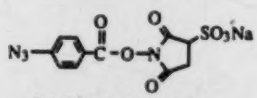
The binding of the potential photo-affinity labeled derivatives (**12i**, **12j**) with recombinant GlcNAcT-V is under investigation.

2.8 Conclusions

All of the trisaccharide analogs (**11a-11k**, **12a-12j**) are competitive inhibitors of the acceptor substrate **5** (K_m = 29 μM) despite the very large variations in molecular structure at or near the position normally transferred to by the enzyme. The fact that such a

large variety of substitutions are tolerated at C-6' or C-4' of the inhibitory trisaccharides indicates that in forming the E-I (or E-S) complexes, neither the potentially reactive OH-6' nor the neighboring OH-4' makes important contacts with the enzyme, and that the α-Man residue to which GlcNAcT-V transfers is not tightly bound by the enzyme prior to transfer. Some of the modifications at C-6' and C-4' increase the inhibitor activities, but the analog with a bulky positively charged group at C-6' (**11h**) and the analog with bulky group (**11j**) at C-6' showed lower activity. Though no clear structure-activity relationship is evident, the results do suggest, however, that the residue with a positive charge near the site of transfer results in unfavorable interactions while hydrophobic or anionic groups are better tolerated.

Table II. Evaluations of Trisaccharides **12a-12j** and **13** as Inhibitors of GlcNAcT-V[a]

Compound	R—	Reagents Used for Amino Derivatization	K_i (μM) I	K_i (μM) II
12a	H—		106	95
12b	CH_3CO—	Ac_2O / MeOH	20	24
12c	C_6H_5CO—	i. C_6H_5COCl / Py ii. NaOMe / MeOH	10	3
12d	$Na^+ \cdot OOC$—CH_2CH_2—CO—	i. succinic anhydride / MeOH ii. $NaHCO_3$ aq.	6	8
12e	ICH_2CO—	$(ICH_2CO)_2O$ / MeOH	14	18
12f	CH_2=CH—CO—	CH_2=$CHCOCl$ / DMF / $NaHCO_3$	13	25
12g		i. $(ICH_2CO)_2O$ / MeOH ii. C_6H_5SH / $NaHCO_3$ aq.	20	8
12h		2, 4 - Dinitrofluorobenzene / Phosphate buffer	60	9
12i		 DMF / 0.1 N aq. $NaHCO_3$	7	
12j		 0.1 N aq. $NaHCO_3$	8	
13			345	

I. Partially purified GlcNAcT-V from hamster kidney.
II. Cloned rat kidney GlcNAcT-V.

[a] Experimental errors ±5%

CHAPTER 3

Synthesis and Evaluation of Thioglycoside Analogs as Acceptors for *N*-Acetylglucosaminyltransferase-V[a,b]

3.1 Introduction

Knowledge of the detailed substrate specificity of GlcNAcT-V is essential for a rational approach to inhibitor design. It has been previously found that a simpler synthetic trisaccharide **3**, a partial structure of **1**, is an effective substrate for the enzyme yielding the expected tetrasaccharide **4** [87]. The enzyme tolerates the substitution of the β-Man residue in **3** by a β-Glc residue as trisaccharide **5** was found to be an excellent acceptor [88]. The enzyme further tolerates the substitution of the terminal GlcNAc moiety by a β-Glc residue, since the trisaccharide **6** was also found to be a good acceptor [89]. The aliphatic aglycon in **3** and **5** was incorporated into these structures in order to facilitate the enzyme assay procedures [90]. In this chapter, we continue these enzyme-specificity studies where the requirement of the natural intra-residue oxygen linkage of **6** in substrate recognition by GlcNAcT-V is assessed through the synthesis and enzymatic evaluation of the two thio-analogs **50** and **51** (Fig. 30) which have an intersaccharidic sulfur atom. Another thio-analog (**52**) (Fig. 30) which has a free thiol group attached to the 2 position of the terminal Glc unit by an ethylene linkage was also synthesized and enzymatically evaluated. The enzymatic experiment results provide important information for the design of GlcNAcT-V inhibitors.

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[a] The enzyme inhibition experiments reported in this chapter were performed by Dr. Hong Li and Ms. Catharine A. Compston in the group of Prof. Monica M. Palcic.

[b] Part of this work is *in press* in the *Canadian Journal of Chemistry*.

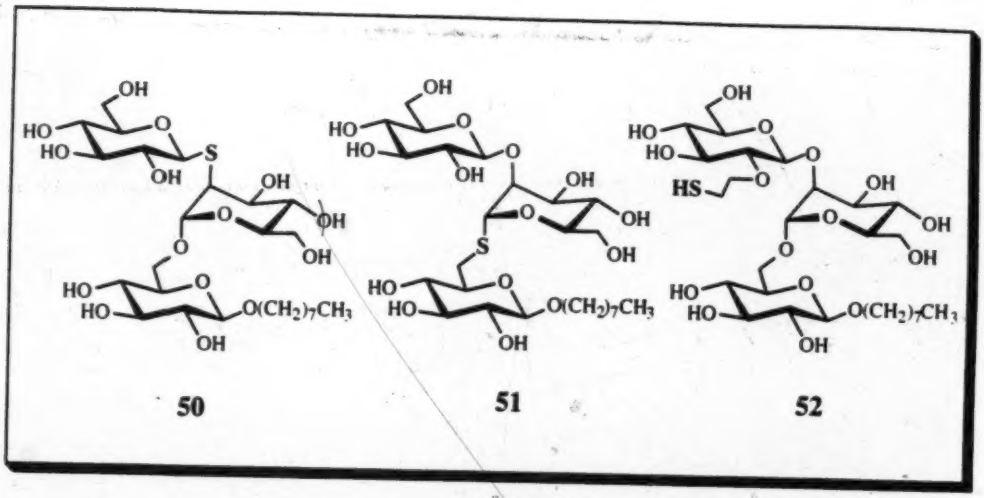


Fig. 30. Structures of thioglycoside analogs **50**, **51** and **52**

3.2 Chemical Synthesis of Thioglycoside 50

The retrosynthetic analysis for the preparation of **50** is shown in Fig. 31. In designing the synthesis of **50**, we chose 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (**54**) as the central building block. The opening of the epoxide ring in **54** by alcohol **16** was expected to produce an α/β mixture of disaccharides with OH-2' free. After conversion of OH-2' into a leaving group, S_N2 displacement using a protected 1-thio-D-glucose (**53**) was then envisioned to produce the desired trisaccharide.

Compound **53** was prepared by the reflux of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**17**) with thiourea in acetone to give **55** [125], followed by treatment with potassium metabisulfite ($K_2S_2O_5$) (Fig. 32).

Compound **54**, in turn, was prepared by direct stereoselective epoxidation of tri-*O*-benzyl-D-glucal with dimethyldioxirane [126, 127] according to Danishefsky's method [128].

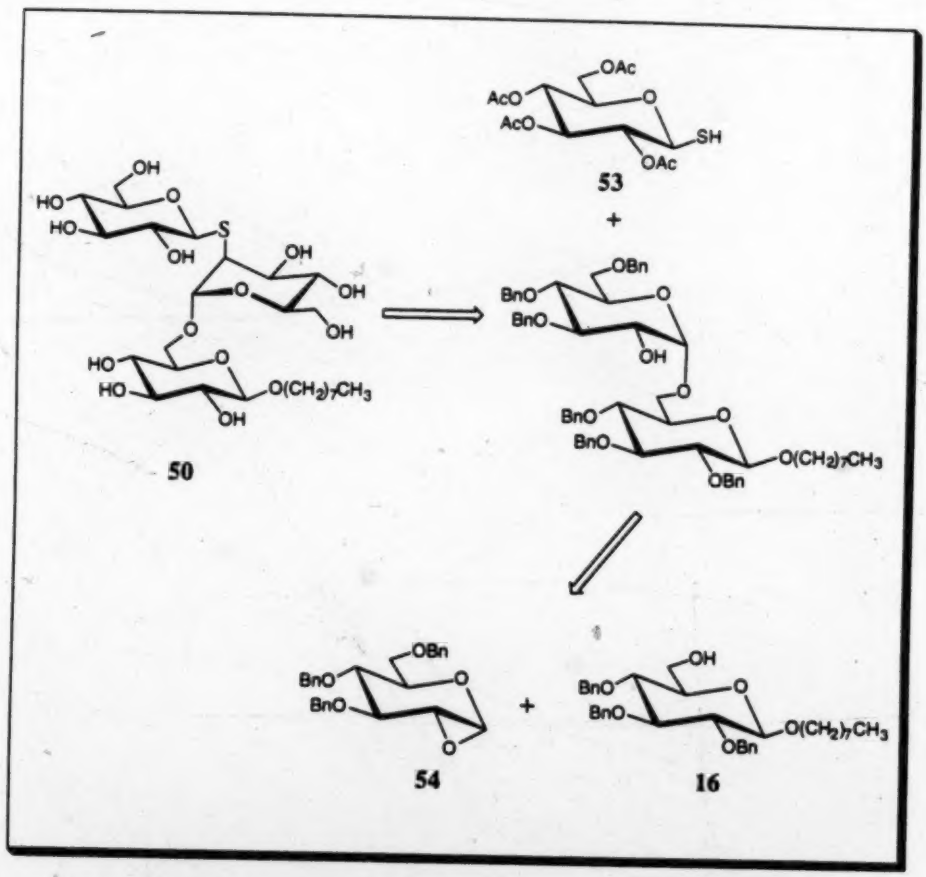


Fig. 31. Synthetic strategy for the preparation of thioglycoside 50

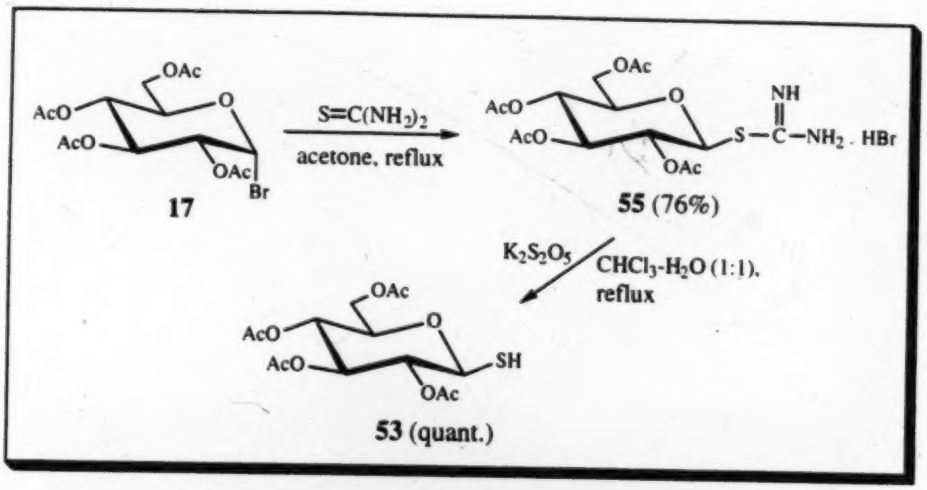


Fig. 32. Preparation of building block **53**

The coupling of **54** with alcohol **16** was carried out in THF at -78 °C using trifluoromethanesulfonic acid as catalyst to produce the desired α-linked disaccharide **56** along with the β-linked disaccharide **57** in 24% and 47% yield, respectively (based on consumed alcohol). Treatment of **56** with excess trifluoromethanesulfonic anhydride in anhydrous pyridine gave the 2'-triflate (**58**) in quantitative yield. The S_N2 displacement of **58** was carried out by using the sodium salt of the 1-thio-D-glucose derivative (**53**) in DMF. The sodium salt was made by treating **53** with sodium hydride in THF. The trisaccharide **59** was obtained in 25% yield. Zemplén deacetylation of **59** yielded **60**. Final debenzylation of **60** by treatment with Na / liq. NH_3 afforded the target trisaccharide **50** in 79% yield (Fig. 33).

Compound **50** was characterized by ^1H NMR spectrocopy, including a ^1H-^1H 2D COSY experiment, ^{13}C NMR spectroscopy (APT), HMQC and high resolution FAB mass spectrometry.

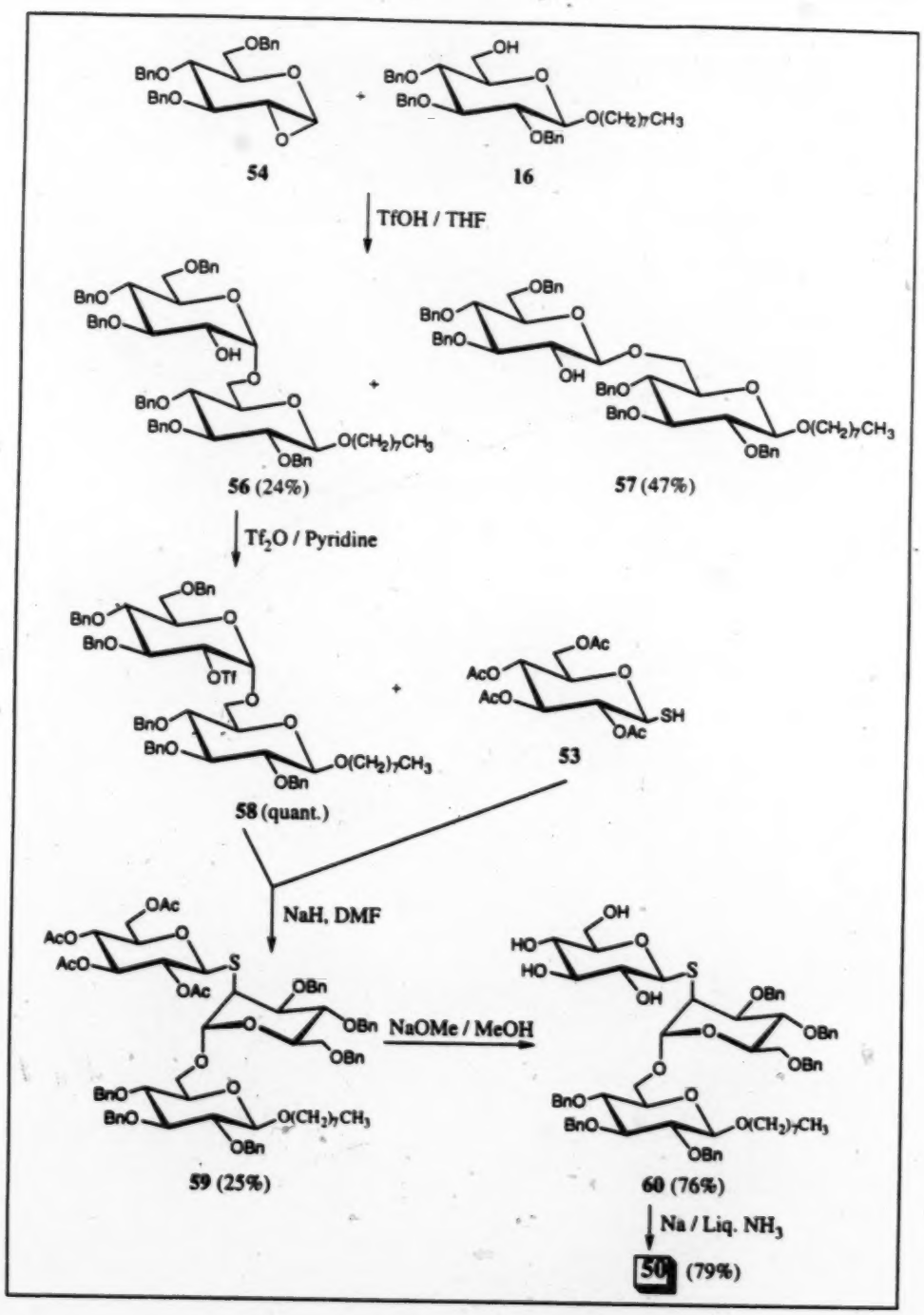


Fig. 33. Synthesis of thioglycoside **50**

3.3 Chemical Synthesis of Thioglycoside 51

The retrosynthetic analysis of **51** is shown in Fig. 34. In designing the synthesis of **51**, we chose 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannopyranose (**61**) as the central building block. The opening of the epoxide **61** by highly nucleophilic **62** would provide a disaccharide with OH-2' free for further coupling to give the desired trisaccharide.

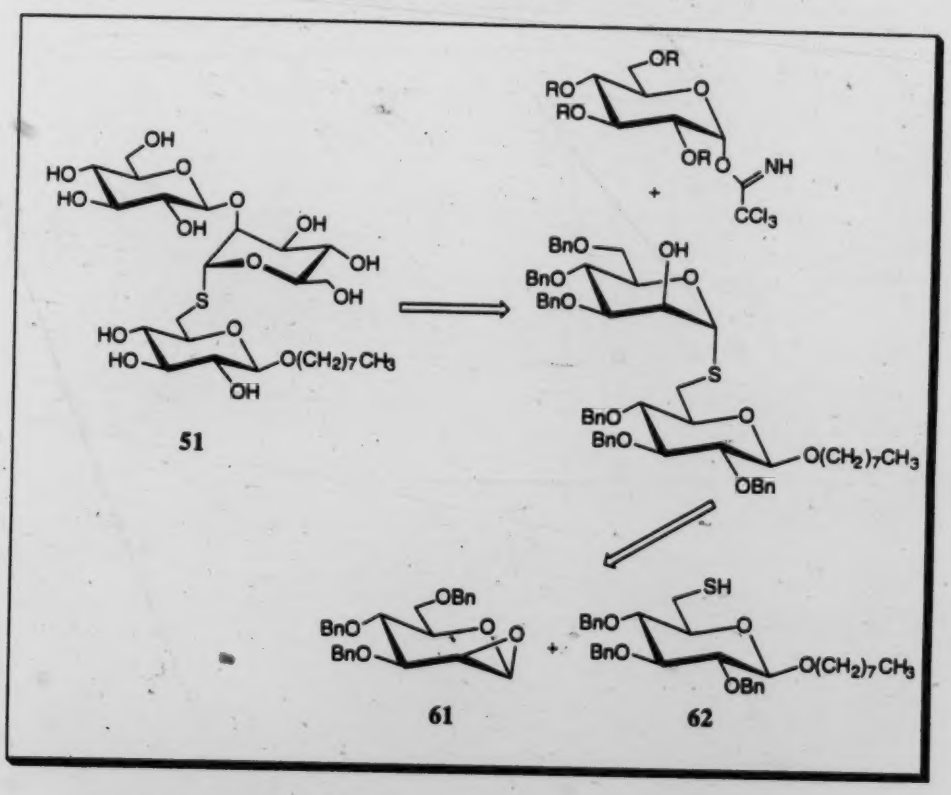


Fig. 34. Synthetic strategy for the preparation of thioglycoside **51**

Epoxide **61** was prepared from 3,4,6-tri-*O*-acetyl-D-mannopyranose 1,2-(methyl orthoacetate) (**63**) [129] according to published procedures. Deacetylation of **63** followed by benzylation gave the benzylated orthoester **64**. Hydrolysis of **64** provided 3,4,6-tri-*O*-

benzyl-D-mannopyranose (**65**) [130]. Treatment of **65** with hydrogen chloride in ether followed by work up with NH_3 afforded epoxide **61** [131] (Fig. 35).

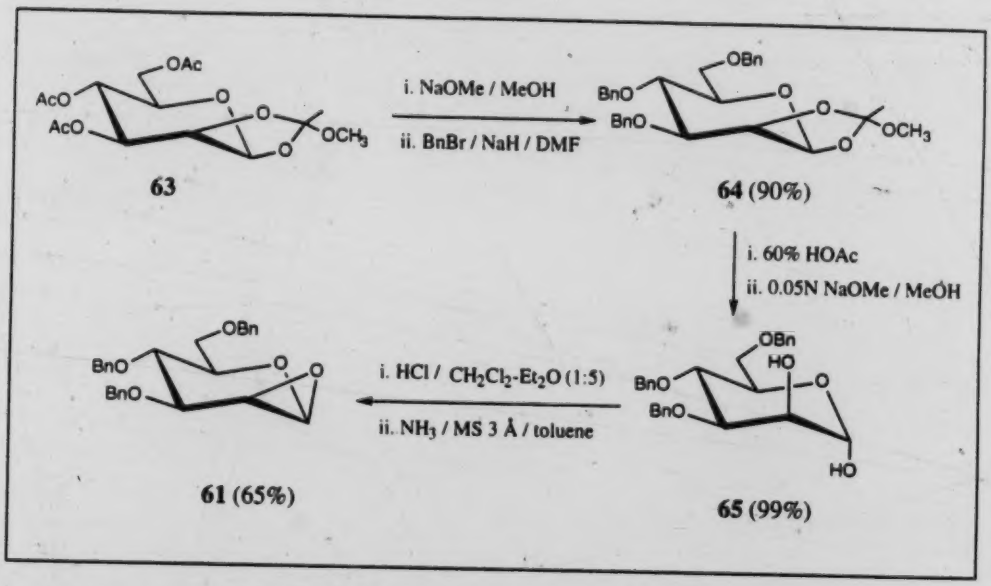


Fig. 35. Preparation of 1,2-anhydro-3,4,6-tri-*O*-benzyl-β-D-mannopyranose (**61**)

Thiol **62** was prepared from **16** by mesylation followed by reaction with potassium thioacetate and deacetylation (Fig. 36).

The required imidate **71** was prepared by treatment of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**47**) [123] with silver carbonate in wet acetone [132] to give hemiacetal **70**, followed by its reaction with trichloroacetonitrile and sodium hydride in dichloromethane [133]. Compound **70** [123] was prepared from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide *via* orthoesters **68** [134] and **69** (Fig. 37).

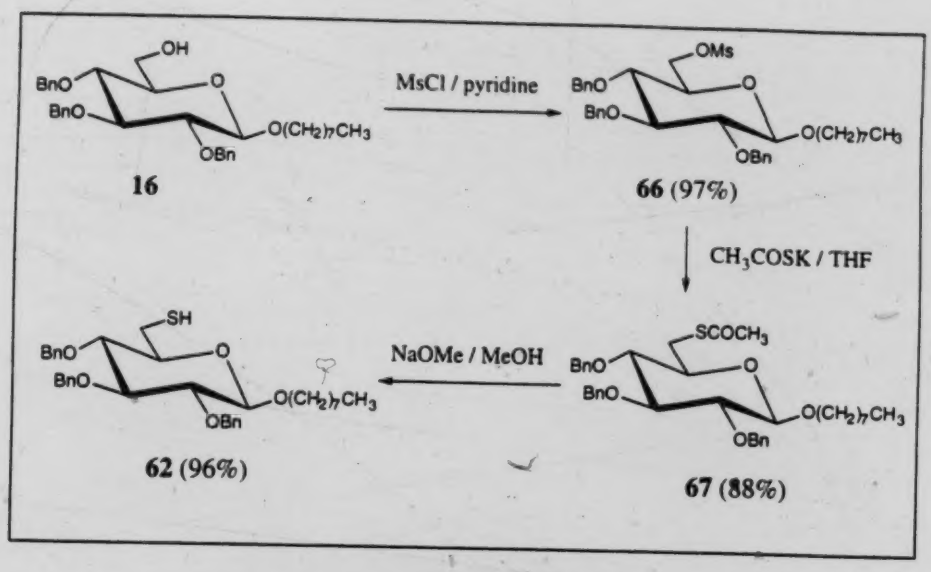


Fig. 36. Preparation of building block **62**

The opening of the epoxide **61** by the sodium salt of **62** gave α-linked disaccharide **72** (63%) with OH-2' unprotected for subsequent coupling. Attempts to couple **72** with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopuranosyl)-trichloroacetimidate [133], using either $BF_3 \cdot Et_2O$ or TMSOTf as promoter, were unsuccessful. When *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-trichloroacetimidate [133] was used as donor, the coupling gave an α/β mixture (ratio *ca.* 9:1) which could not be resolved on TLC. Finally, using *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl)-trichloroacetimidate (**71**) as donor, glycosylation of **72** afforded the desired β-linked trisaccharide **73** in 88% yield. After Zémplen deacetylation (92%), debenzylation with Na / liq. NH_3 furnished the target trisaccharide (**51**) in 82% yield (Fig. 38).

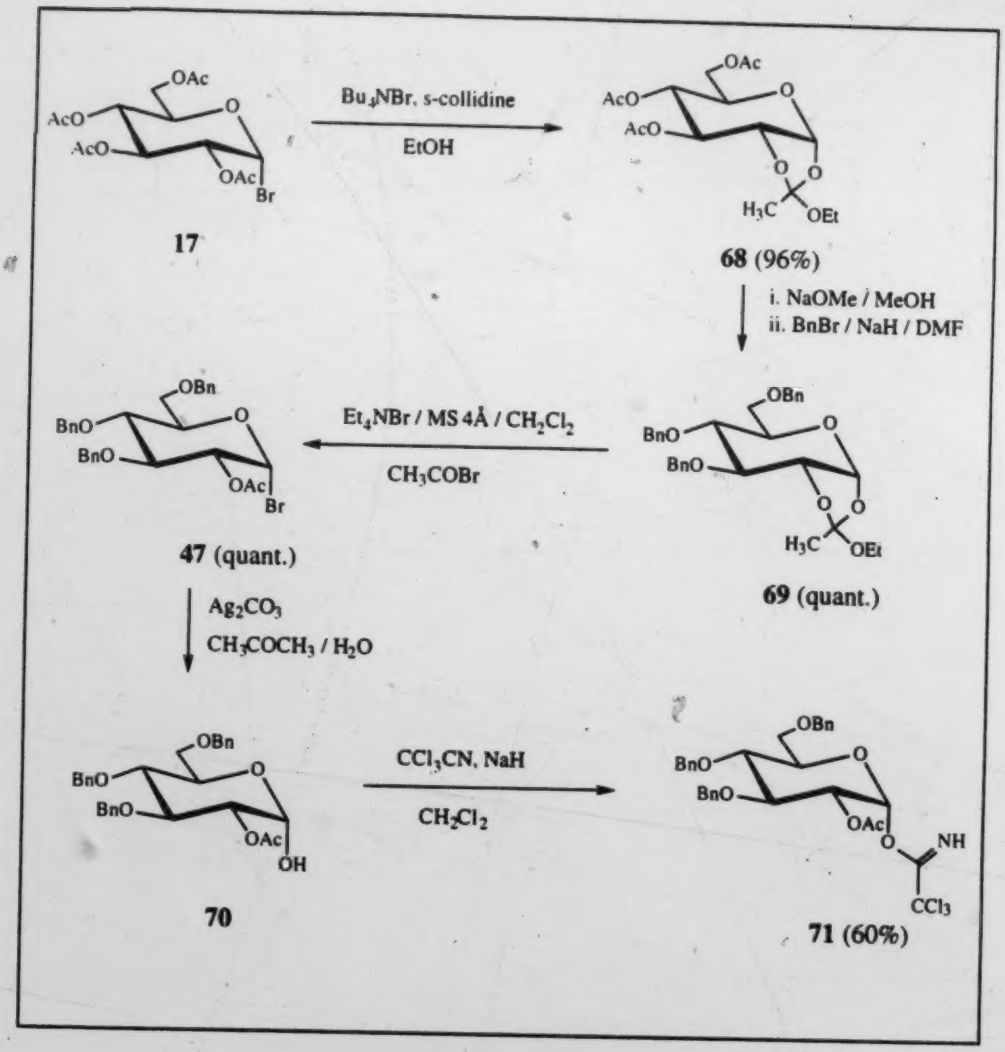


Fig. 37. Preparation of imidate **71**

Compound **51** was characterized by ^{1}H NMR spectroscopy, ^{1}H-^{1}H 2D COSY, ^{13}C NMR (APT), HMQC and high resolution FAB mass spectrometry.

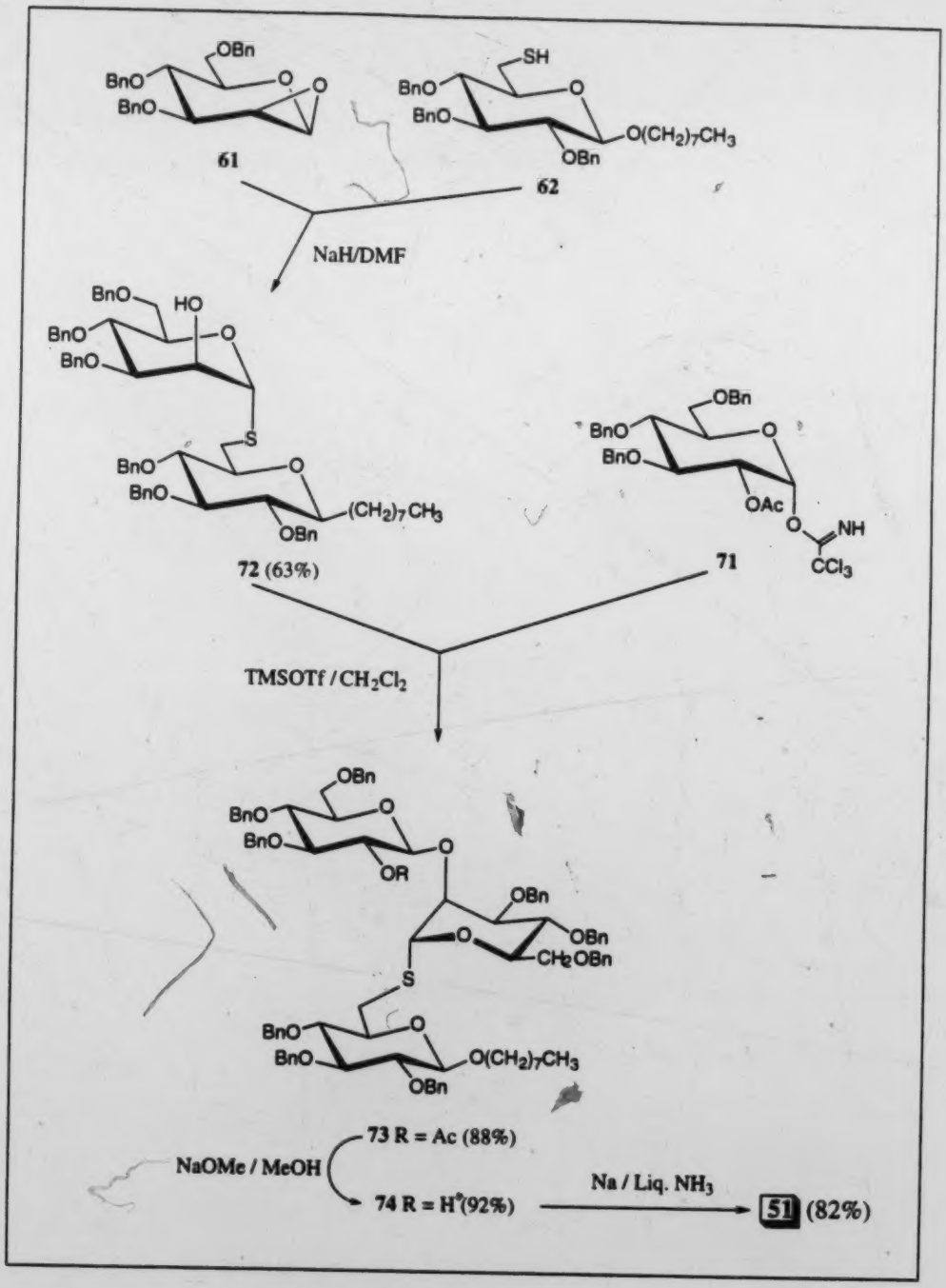


Fig. 38. Synthesis of thioglycoside **51**

3.4 Chemical Synthesis of Thioglycoside 52

Trisaccharide **79**, which has a OH-2" free, was used for the synthesis of **52**. The preparation of **79** is shown in Fig. 39. Coupling of alcohol **16** with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl bromide (**75**) gave disaccharide **76** in 85% yield. Compound **75** was prepared by treatment of orthoester **64** with tetraethylammonium bromide and acetyl bromide [123]. After deacetylation of **76** to give alcohol **77**, glycosylation with **47** gave the desired trisaccharide **78** in 55% yield. Deacetylation of **78** provided **79**.

Treatment of **79** with allyl bromide and sodium hydride in DMF gave the 2"-*O*-allyl trisaccharide in 81% yield. Ozonolysis, followed by reduction with sodium borohydride, produced **81** with the required hydroxyethyl group at O-2" (65%). Mesylation of **81** followed by displacement with potassium thioacetate gave **83** in 86% yield. Deprotection of **83** with Na / liq. NH_3 afforded the target compound **52**.

3.5 Enzymatic Evaluations of Trisaccharide Analogs 50-52 as Acceptors for GlcNAcT-V.

Compounds **50**, **51** and **52**, and the unmodified structure **6**, were kinetically evaluated as acceptors for GlcNAcT-V using the cloned rat kidney enzyme [84]. The enzyme experiments were performed according to a well-established radioactive "Sep-Pak assay" method [90, 93 and 94]. The results of the kinetic evaluations are reported in Table III. Both compounds **50** and **51** remained relatively good substrates for GlcNAcT-V. The V_{max} values are 2 to 3 fold higher than their parent compound (**6**), however, their binding is likely weaker as reflected in the higher K_m values for these analogs. Trisaccharide **51** was also evaluated using GlcNAcT-V isolated from hamster kidney [94, 95, 108]. As was

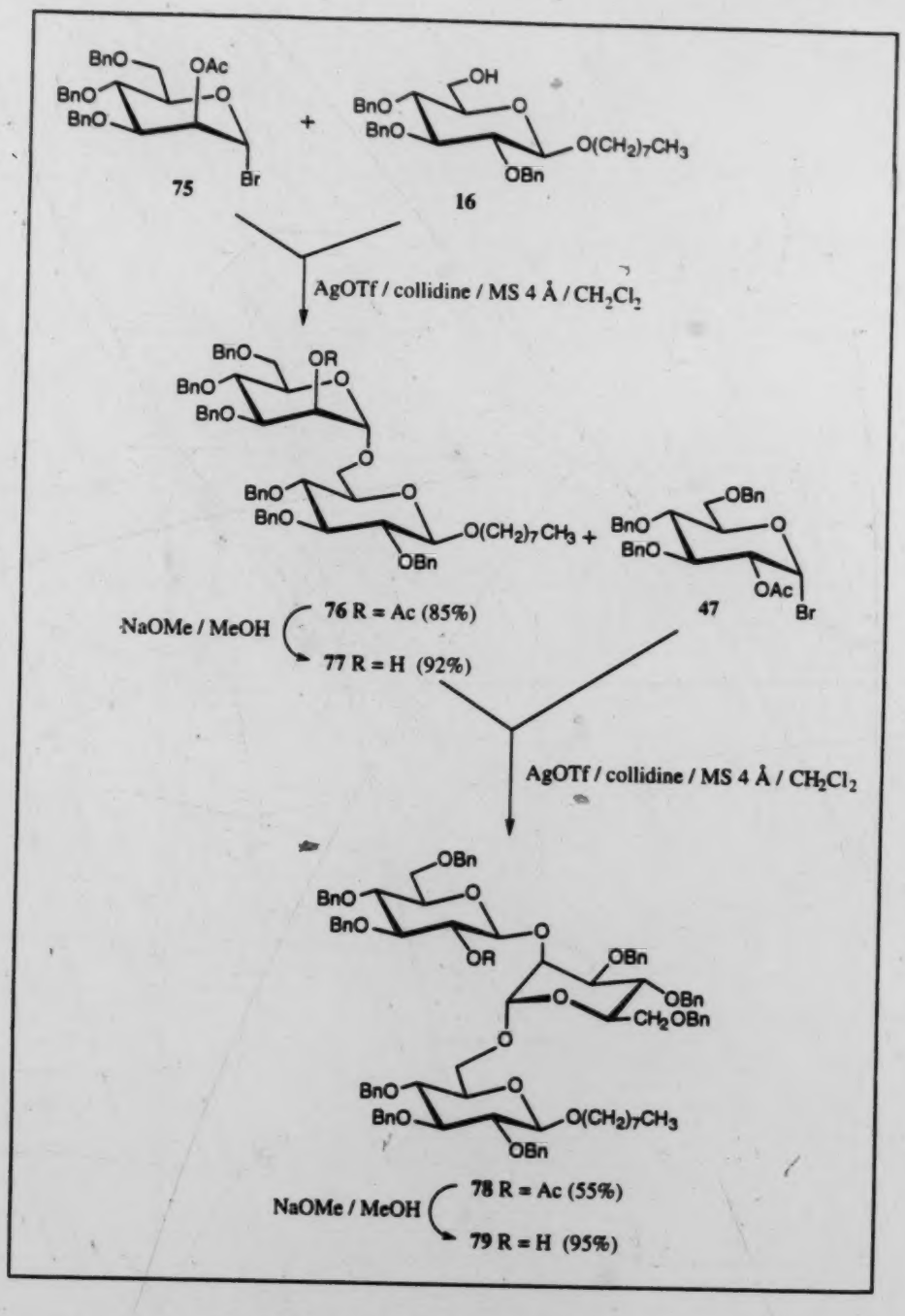


Fig. 39. Synthesis of trisaccharide **79**

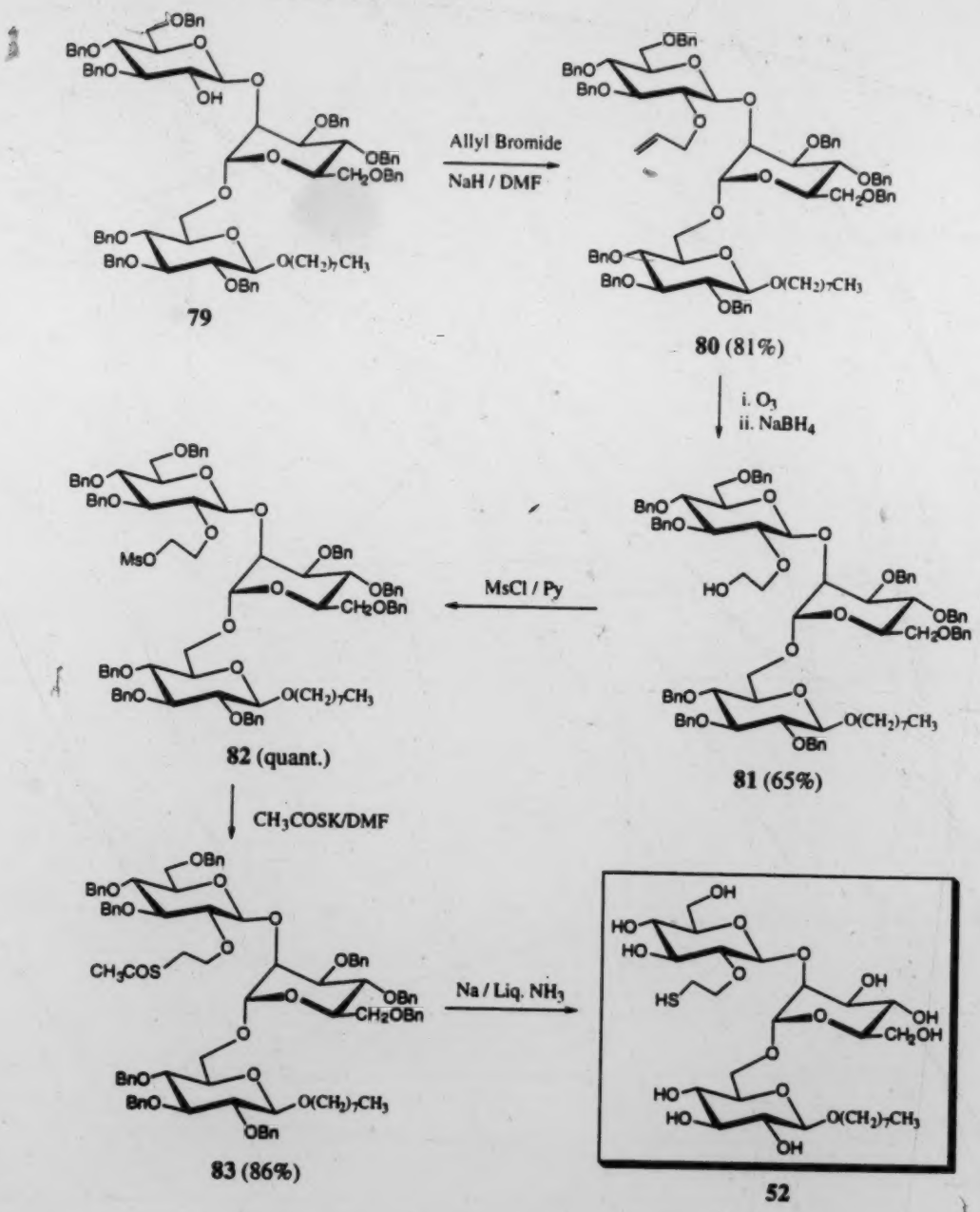


Fig. 40. Synthesis of trisaccharide **52**

previous found [98], both sources of GlcNAcT-V have essentially identical substrate specificity. Trisaccharide **52** was found to be a poor acceptor for GlcNAcT-V with a high K_m value, although its V_{max} is comparable to the values of good substrates.

Table III. Kinetic Parameters for GlcNAcT-V Acceptor Analogs 50 and 51

Acceptor	GlcNAc T-V	K_m (μM)	V_{max} (pmol/min)	$V_{max, rel}$[a]
6	cloned	111±7	2.1	100
50	cloned	376±16	3.5	167
51	cloned	300±24	6.1	290
51	isolated	256±12	9.0	—
52	isolated	7800±1800	2.9	—

[a] The value for compound **6** is arbitrarily set to 100.

3.6 Preparative Enzymatic Synthesis of Tetrasaccharides 84 and 85

To confirm that the thioglycosides **50** and **51** were indeed acceptors for GlcNAcT-V, preparative enzymatic syntheses were performed. When the acceptors were incubated with UDP-GlcNAc and GlcNAcT-V, the enzyme transferred a β-D-GlcNAc residue to OH-6 of the core D-Man*p* unit of the acceptors, converting **50** and **51** into the expected tetrasaccharides **84** and **85** respectively. Tetrasaccharides **84** and **85** were characterized by means of their ^{1}H NMR spectra and FAB mass spectra (Fig. 41).

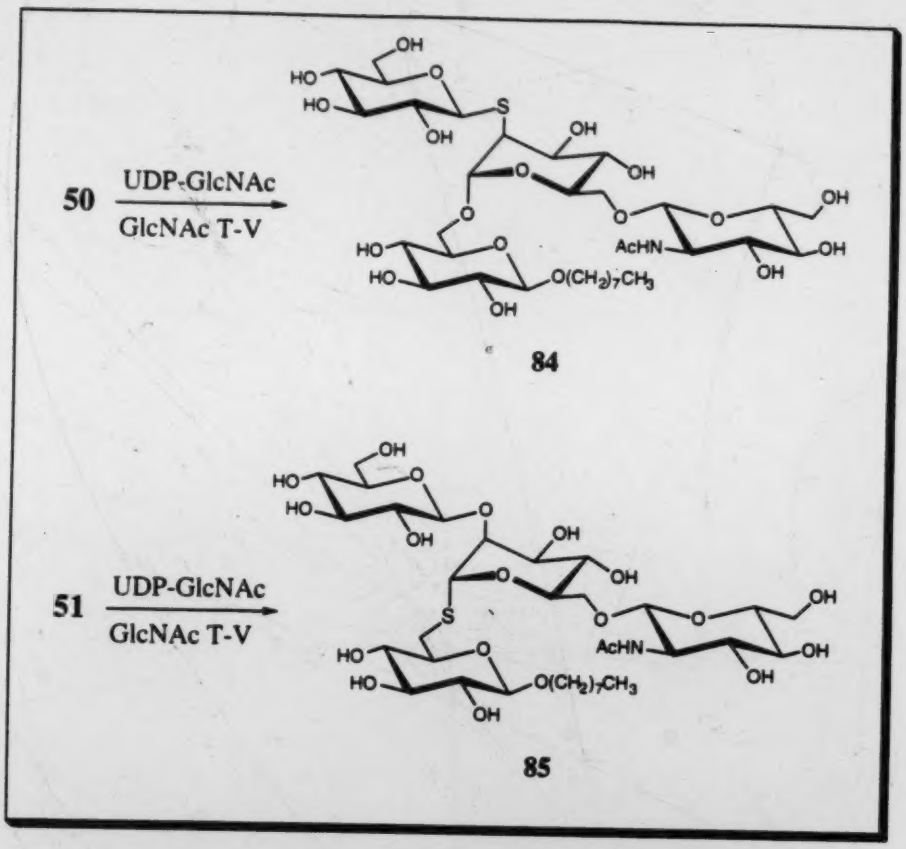


Fig. 41. Enzymatic synthesis of tetrasaccharides **84** and **85**

3.7 Conclusion

This work indicates that neither of the intersaccharidic oxygen atoms in the core trisaccharide acceptor is involved in specific recognition by GlcNAcT-V, since the enzyme tolerates the substitution of the natural intersaccharidic oxygen linkages by sulfur. This information is very useful for the design of specific inhibitors for GlcNAcT-V. Since thioglycosides are resistant to cleavage by glycosidases, these structures should prove useful as metabolically stable acceptors for assaying GlcNAcT-V.

CHAPTER 4

Synthesis and Conformational Analysis of Two Diastereospecifically Deuterated Derivatives of β-D-GlcNAc-(1→2)-α-D-Man-(1→6)-β-D-Glc-O$(CH_2)_7CH_3$

4.1 Introduction

It is generally acknowledged that knowledge of the conformations of oligosaccharides is important for understanding their interactions with proteins (enzymes, antibodies, lectins, receptors, etc.) [135-138]. Oligosaccharides involved in the interactions are mainly made up of hexopyranose units that exist in chair conformations. The overall oligosaccharide conformation is then controlled by the rotation of the glycosidic linkages and the bonds to the exocyclic groups such as hydroxymethyl groups and *N*-acetyl groups. The conformation of the hydroxymethyl group is of interest, because this hydroxy group may be involved in binding, and furthermore, the conformation of the C5-C6 bond determines the overall shape of oligosaccharides containing (1→6) linkages [139].

The conformation of the hydroxymethyl group in hexopyranoses is predominantly determined by the rotation around the C5-C6 linkage [140] as described by the dihedral angle ω (O5-C5-C6-O6) for the hexopyranoses. In principle the ω angle can be any value from 0° to 360°, but the hydroxymethyl groups are viewed as existing predominately in the three most stable staggered conformations (gg, gt, tg) (Fig. 42). Determination of the rotameric distribution about the C5-C6 bond has been investigated by using semi-empirical energy calculations [141], X-ray diffraction [142], chiroptical methods [143] and especially NMR spectroscopy [139].

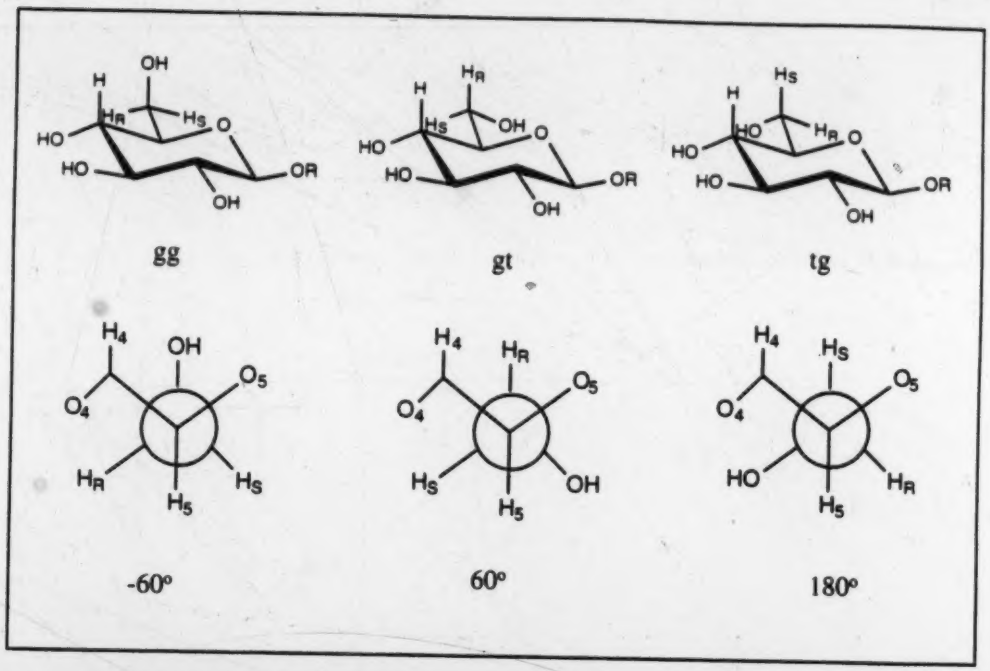


Fig. 42. Nomenclature of the three staggered conformations for the hydroxymethyl group of D-glucopyranoside

NMR spectrocopy is the most useful technique for the investigation of the hydroxymethyl group conformation. Information about the conformation of hydroxymethyl groups can be obtained from NMR spectroscopy using the vicinal coupling constants between the H-5 and the two H-6 protons. The relationship between vicinal ^1H-^1H coupling constants and the relative orientations of the two H-6 protons can be described by empirical equations which are denoted as "Karplus-equations" after the first formulation by Karplus [144].

The two methylene protons at C-6 of D-hexopyranoses (H-6*pro*R and H-6*pro*S, denoted as H6R and H6S) are not magnetically equivalent and give separate signals in their high-resolution ^1H-NMR spectrum since they are diastereotopic. In a conformational study of the exocyclic C5-C6 bonds of D-hexopyranoses or the (1→6)-linkage of their

oligosaccharides, an unambiguous assignment of these two protons is crucial. In early studies, this assignment was generally based on the prediction of the relative chemical shifts of H6R and H6S by empirical rules that were not always correct. About a decade ago, H. Ohrui and his coworkers developed a general method for the preparation of stereospecifically C-6-deuterium-labeled hexopyranosides [145-147]. This method involved a highly stereoselective photo-bromination [48, 149] of 1,6-anhydro-per-*O*-benzoyl-β-D-hexopyranoses to give C-6exo bromides and subsequent reduction with tri-*n*-butyltin deuteride to afford (6*S*)-deuterated 1,6-anhydropyranoses. After debenzoylation and acetolysis, stereospecifically deuterated monosaccharides were obtained. Stereospecifically deuterium-labeled sugars allow the unambiguous assignment of H6R and H6S in NMR, and greatly facilitates the conformational analysis of hydroxymethyl groups in carbohydrates [150-160].

The trisaccharide β-D-GlcNAc-(1→2)-α-D-Man-(1→6)-β-D-Glc-*O*-$(CH_2)_7CH_3$ (**5**) [88] is a synthetic acceptor substrate for the enzyme *N*-acetylglucosaminyltransferase-V (GlcNAcT-V) which transfers an additional β-GlcNAc residue to the 6-position of the central α-Man unit. A systematic analysis of the recognition of oligosaccharide substrate by GlcNAcT-V would be greatly enhanced by the detailed knowledge of the conformational properties of trisaccharide **5**. Trisaccharide **5** is known to be conformationally labile about the α-Man-(1→6) linkage. Although two conformationally restricted trisaccharide analogs of **5** have been synthesized and enzymatically evaluated as substrates for GlcNAcT-V [91], little direct information was obtained about the rotameric distribution about the C5-C6 bond of the α-Man-(1→6) linkage due to the complexity of the NMR spectra and the difficulty in assigning the H6R and H6S signals unequivocally.

In this chapter, the synthesis of two trisaccharides, which are stereospecifically labeled with deuterium (**5R**, **5S**) (Fig. 43) are reported. Therefore, the signals for H6R

and H6S of the Glc residue could be assigned unambiguously. These two compounds will permit the study of the rotameric distribution about the C5-C6 bond at the α-Man-(1→6) linkage of the trisaccharide acceptor and possible conformation changes on binding to recombinant GlcNAcT-V using NMR techniques.

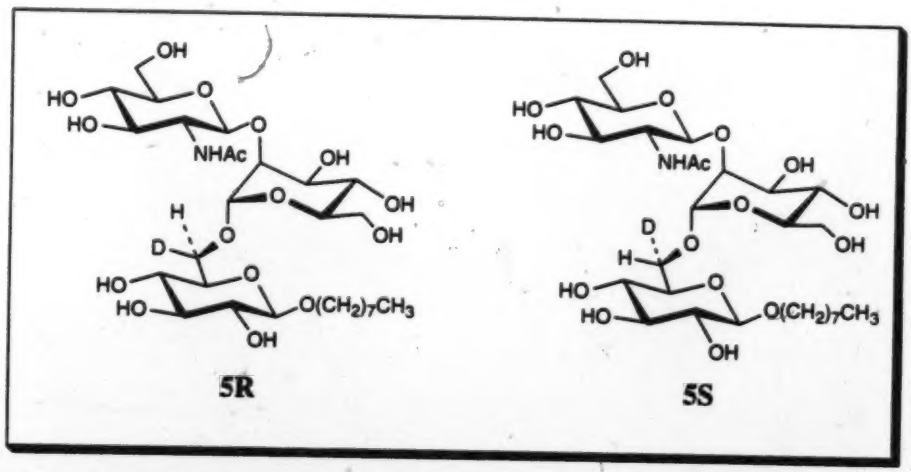


Fig. 43. Two stereospecifically deuterium labeled trisaccharide acceptor **5R** and **5S**

4.2 Synthesis of Diastereoselectively Deuterated Trisaccharides

The key intermediates for the synthesis of the two diastereospecifically deuterated trisaccharides (**5S**, **5R**) are (6*S*) - [6-^{2}H] octyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**96S**) and (6*R*) - [6-^{2}H] octyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**96R**). Compound **96S** was synthesized from (6S) - [6-^{2}H] 1,2,3,4,6-penta-*O*-acetyl-α-D-glucose (**90**) where C6 was stereospecifically deuteriated yielding S configuration at C-6. Compound **90** was prepared from 1,6-anhydro-2,3,4-tri-*O*-benzoyl-β-D-glucopyranose (**86**) using Ohrui's method [145] (Fig. 44).

Treatment of **90** with 30% HBr in acetic acid gave (6S) - [6-^{2}H] 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **91**. The reaction of bromide **91** with *n*-octanol in the presence of silver oxide, followed by deacetylation afforded (6*S*) - [6-^{2}H] octyl β-D-glucoside (**93**) in 62% yield. Reaction of **93** with chloro(4-methoxyphenyl)-diphenylmethane (MMTCl) gave **94**, which was directly benzylated to provide **95**. Treatment of **95** with aqueous acetic acid provided **96S** (64% yield in three steps) (Fig. 45).

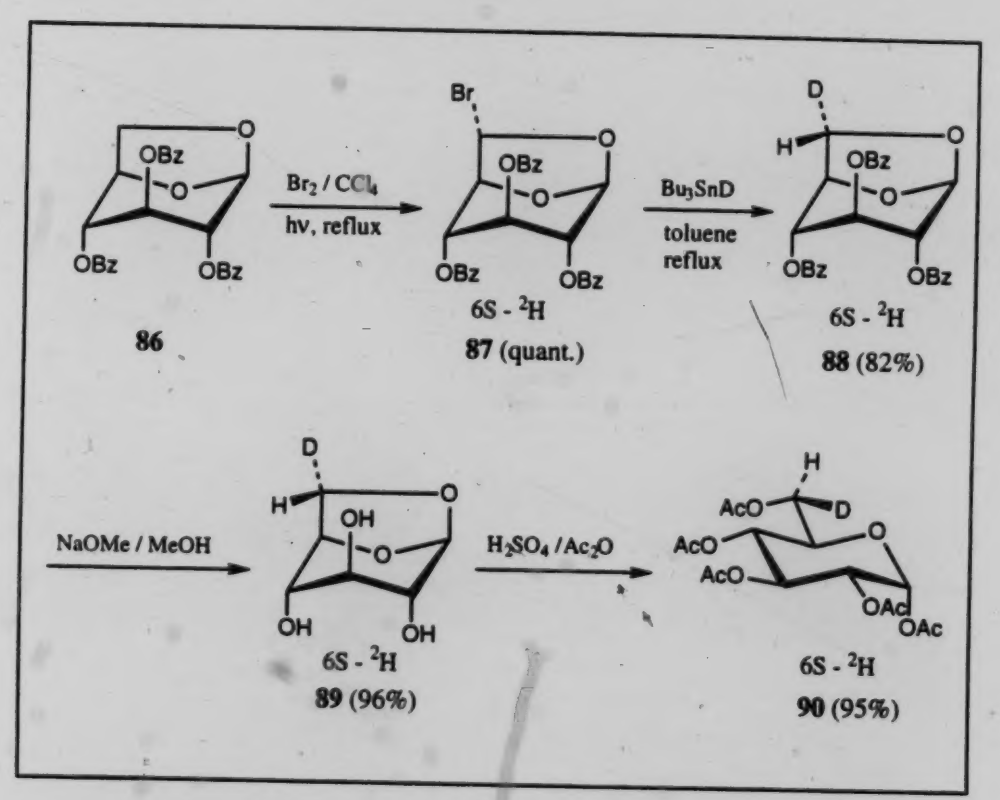


Fig. 44. Preparation of 6*S*-[6-^{2}H]-1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**90**)

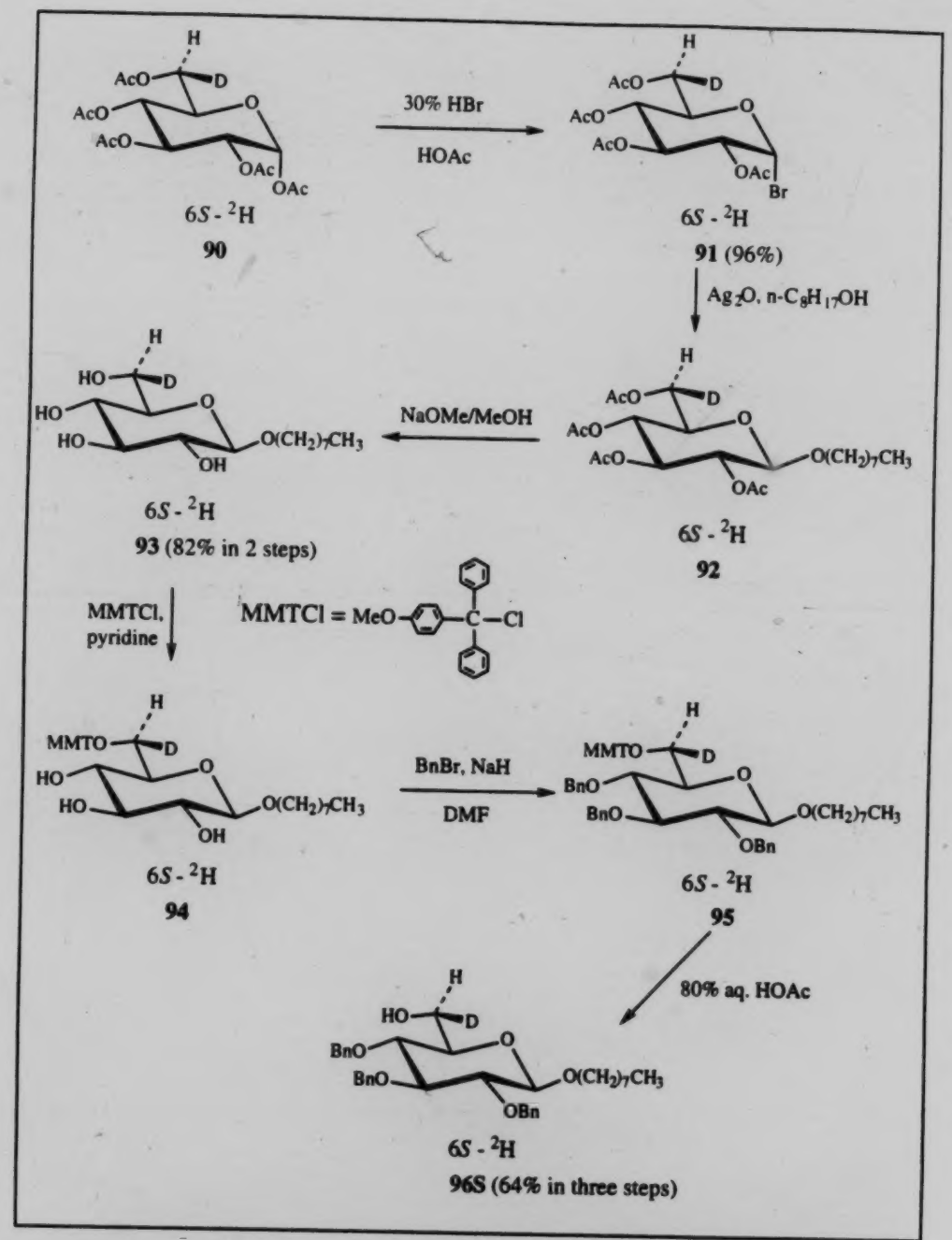


Fig. 45. Synthesis of stereospecifically deuterium-labeled building block **96S**

The corresponding 6-(*R*)-deuterium-labeled intermediate **96R** was prepared by treatment of **96S** under Mitsunobu conditions using benzoic acid, triphenylphosphine and diethyl azodicarboxylate [152, 153], giving the 6-benzoate (**97**), with the inverted configuration at C6, in 92% yield. Debenzoylation of **97** gave **96R** in 95% yield (Fig. 46).

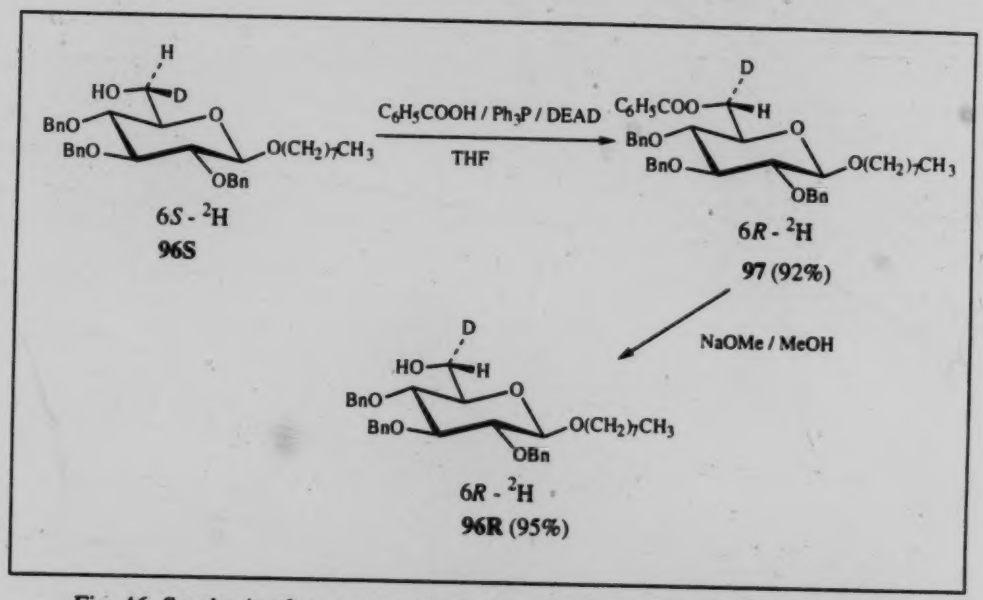


Fig. 46. Synthesis of stereospecifically deuterium labeled building block **96R**

The coupling of **96S** or **96R** with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl bromide (**75**) promoted by AgOTf gave α-linked disaccharide **98S** or **98R**, respectively. Deacetylation of **98S/98R** produced **99S/99R** bearing free OH-2' groups for further coupling. Glycosylation of **99S/99R** with imidate **100** afforded the desired trisaccharides **101S/101R**, respectively. Removal of the *N*-phthalimido and *O*-acetyl groups of **101S/101R**, and subsequent acetylation with acetic anhydride in pyridine gave **102S/102R**. Zemplén deacetylation followed by hydrogenolytic cleavage of the benzyl groups catalyzed by $Pd(OH)_2$ afforded the target trisaccharides **5S/5R** (Fig. 47).

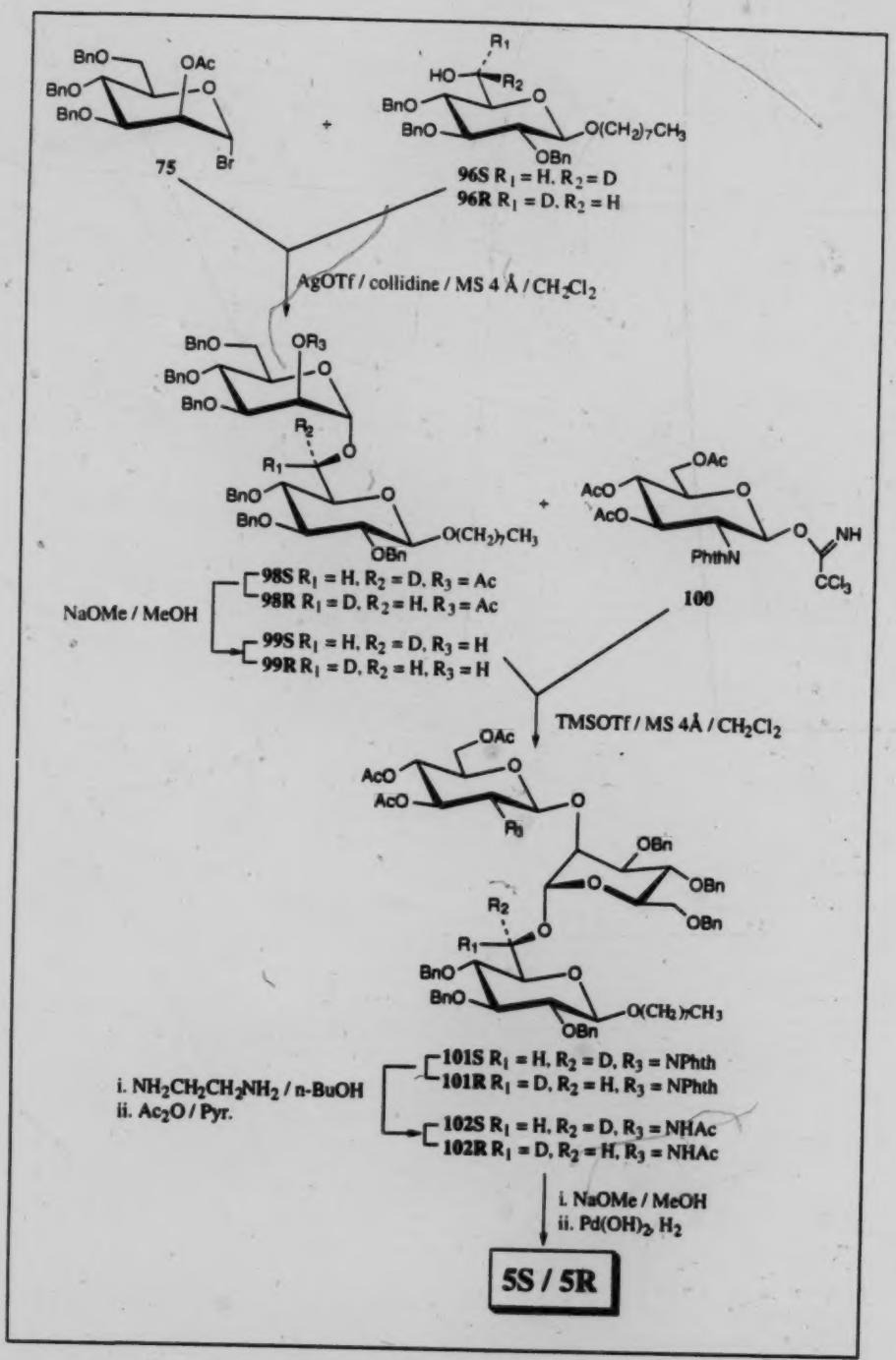

75
96S R1 = H, R2 = D
96R R1 = D, R2 = H
AgOTf / collidine / MS 4 Å / CH2Cl2
98S R1 = H, R2 = D, R3 = Ac
98R R1 = D, R2 = H, R3 = Ac
NaOMe / MeOH
99S R1 = H, R2 = D, R3 = H
99R R1 = D, R2 = H, R3 = H
100
TMSOTf / MS 4Å / CH2Cl2
101S R1 = H, R2 = D, R3 = NPhth
101R R1 = D, R2 = H, R3 = NPhth
i. NH2CH2CH2NH2 / n-BuOH
ii. Ac2O / Pyr.
102S R1 = H, R2 = D, R3 = NHAc
102R R1 = D, R2 = H, R3 = NHAc
i. NaOMe / MeOH
ii. Pd(OH)2, H2
5S / 5R

Fig. 47. Synthesis of the two stereospecifically labeled trisaccharides **5S** and **5R**

4.3 Conformational Analysis of Stereospecifically Deuterated Trisaccharides 5R and 5S

Unequivocal assignment of H6R and H6S is essential for a ^{1}H NMR study to determine the conformations about the C5-C6 bond in trisaccharide **5**, because the $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ values provide the key information on the rotameric distributions. The ^{1}H NMR spectra of the two diastereospecifically deuterated trisaccharides in deuterium oxide were compared with the ^{1}H NMR spectrum of their nondeuterated analog (**5**) (Fig. 48). In the spectrum of **5S**, two doublets of doublets at 3.96 and 3.76 ppm had disappeared, being replaced by a new doublet signal at 3.94 ppm. Since a H6S of **5S** was selectively replaced with a deuterium, the missing signal at 3.76 ppm can be assigned with confidence to H6S, and the signal at 3.94 ppm is assigned to H6R. Comparison of the ^{1}H NMR spectrum of **5R** with that of **5** reinforced these assignments. Thus, the doublet at 3.94 ppm of **5S** and doublet at 3.74 ppm of **5R** were unambiguously assigned as arising from H6R and H6S, respectively. The coupling constants $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ were found to be 4.9 Hz and 2.0 Hz, respectively, in the ^{1}H NMR spectrum of **5**.

A set of two coupling constants $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ can be used to determine the rotameric distribution about the C5-C6 bond. Since a deuterium has no direct effect on the coupling constants [162], the coupling constants obtained with deuterated derivatives can be considered those of the normal compounds. Here, three types of equations, A, B, and C [139], were employed for the calculations of the time-averaged distributions of the three rotamers. The $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ from the stereoisomers **6S** and **6R** were artificially combined for the calculations.

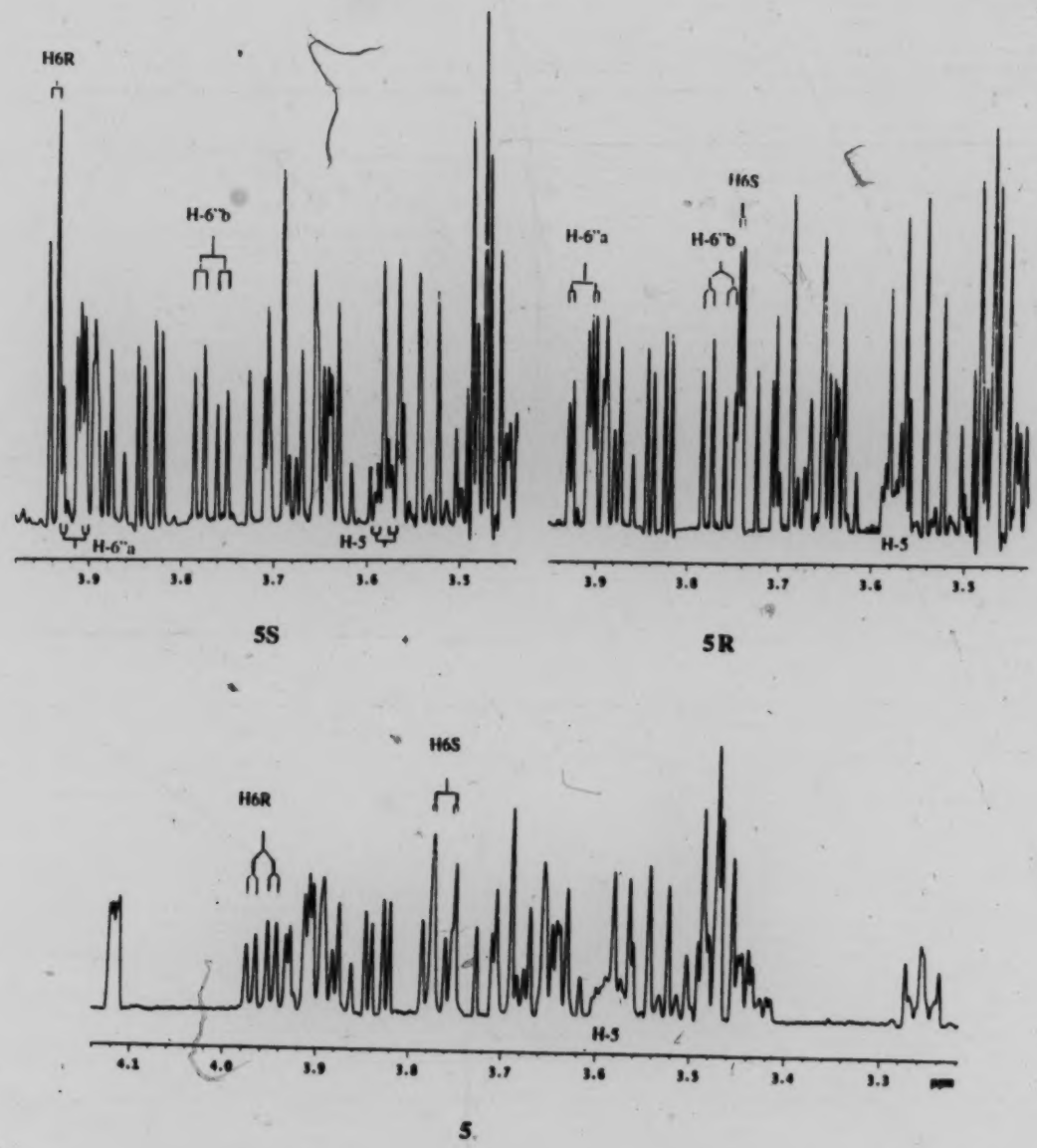


Fig. 48. Partial ^{1}H NMR spectra of trisaccharides 5S, 5R and 5

Table IV. ^{1}HNMR Spectral Data for the H-6*pro*R and H-6*pro*S Signals of 5S / 5R and the Rotameric Distribution About the C5-C6 Bond

No.	chemical shift (ppm)		coupling constant (Hz)		rotamer distribution[a]								
					A[b]			B[b]			C[b]		
	H-6*pro*R	H-6*pro*S	$J_{H5,H6R}$	$J_{H5,H6S}$	gg	gt	tg	gg	gt	tg	gg	gt	tg
5S	3.935		4.9										
5R		3.740		2.0	66	46	-12	67	36	-3	64	34	2

a. Calculated from the $J_{H5,H6R}$ and $J_{H5,H6S}$ values using the Hassnoot-Altona equations with parameters that varied.

b. Equation A: Ar/As = 0.9/2.8, Br/Bs = 10.7/3.1, Cr/Cs = 5.0/2.7

B: Ar/As = 1.7/2.2, Br/Bs = 10.8/2.4, Cr/Cs = 4.1/11.1

C: Ar/As = 1.3/1.3, Br/Bs = 11.5/2.7, Cr/Cs = 5.8/11.7

for general form : $As\ gg + Bs\ gt + Cs\ tg = J_{H5,H6S}$ (1)

$Ar\ gg + Br\ gt + Cr\ tg = J_{H5,H6R}$ (2)

$gg + gt + tg = 1$ (3)

The results indicated that synthetic trisaccharide acceptor **5** of GlcNAcT-V exists predominantly in two conformers, gg and gt about the C5-C6 bond of the α-Man(1→6) linkage in a approximate ratio of 60 and 40, respectively, with a negligibly low population of the tg conformer.

Based on ^{1}H(1D) , ^{13}C(APT), 2D-(G)COSY, 2D-(G)TOCSY, 2D-(T)ROESY, and ^{13}C^{1}H(G)HMQC NMR spectra, the signals of protons and carbons on ^{1}H NMR and ^{13}C NMR spectra were assigned (see experimental section). The rotameric distributions of C5'-C6' of the α-Man residue and C5"-C6" of the β-GlcNAc residue were also calculated (Table V).

Table V. NMR Data and Population of the Three Rotamers About the C5-C6 Bonds for Trisaccharides 5S / 5R

	β-GlcNAc	α-Man	β-Glc
5S			
^{1}H δ (ppm): 6S / 6R	3.92 3.76	3.90 3.63	3.94
J (Hz): $J_{5,6S}$ / $J_{5,6R}$	2.3 5.4	1.8 7.5	4.9
rotamer distributions[b] (gg:gt:tg)	55:45:0	34:66:0	60:40:0[a]
^{13}C δ (ppm): C6	61.5	62.4	66.3
5R			
^{1}H δ (ppm): 6S / 6R	3.91 3.76	3.90 3.63	3.74
J (Hz): $J_{5,6S}$ / $J_{5,6R}$	2.3 5.4	1.8 7.6	2.0
rotamer distributions[b] (gg:gt:tg)	55:45:0	33:67:0	60:40:0[a]
^{13}C δ (ppm): C6	61.5	62.4	66.3

a. Calculated by artificially combining the $J_{H5,H6R}$ and $J_{H5,H6S}$.

b. Calculated by using Haasnoot-Altona equations as follows:

$$0.9\ gg + 10.7\ gt + 5.0\ tg = J_{H5,H6R} \quad (1)$$

$$2.8\ gg + 3.1\ gt + 2.7\ tg = J_{H5,H6S} \quad (2)$$

$$gg + gt + tg = 1 \quad (3)$$

The possible conformation change of trisaccharide acceptors (**5S**, **5R**) on binding to recombinant GlcNAcT-V is going to be investigated using 600 MHz NMR techniques.

CHAPTER 5

Summary and Future Prospects

Increases in the expression of tumor cell-surface carbohydrate structures have been strongly correlated with their metastatic potential, and reversal may lead to a new mode of cancer therapy. The activity of *N*-acetylglucosaminyltransferase-V (GlcNAcT-V), which controls the branching-pattern of asparagine-linked oligosaccharides (*N*-glycans), is correlated with the metastatic potential of lymphoma and other human tumor cells [61, 65, 70-72]. The objective of this thesis research was to provide synthetic probes for GlcNAcT-V that would help define the specificity of the combining site. The long term goal is to develop enzyme inhibitors to be used as potential anti-cancer agents. The following was accomplished:

5.1 New Synthetic Trisaccharide Inhibitors (11a-11k, 12a-12j)

Trisaccharide **11a** and **12a** and a series of their amino-derivatives were successfully synthesized. The enzymatic evaluation of theses analogs demonstrated that the enzyme tolerates a surprisingly wide range of substitutions at C-4 and C-6 on the central Man residue. We conclude that in forming the E-I (or E-S) complexes, neither the potentially reactive OH-6' nor the neighboring OH-4', make important contacts with the enzyme, and the α-Man residue to which GlcNAcT-V transfers is not tightly bound by the enzyme prior to transfer. This information should be of value in the design and development of new inhibitors. It means that the α-Man residue may be omitted in an eventual mimetic structure.

Inhibitors with potential photo-affinity labels were also prepared. These compounds are currently under investigation in the laboratory of Professor M. Pierce at the University of Georgia. They should be useful in attempting to identify active site residues on the enzyme.

5.2 Thioglycosides 50 and 51

Two trisaccharide thioglycosides (**50**, **51**), each having an intersaccharidic sulfur linkage, were found to be efficient acceptors of GlcNAcT-V (**50**, K_m = 376 μM; **51**, K_m = 300 μM). The enzymatic results indicate that GlcNAcT-V tolerates the substitution of the natural oxygen linkage in synthetic acceptor **5** by sulfur. This information should be very useful for the design of GlcNAcT-V inhibitors in the future. In particular, we can take advantage of the nucleophilicity of the thiol group to facilitate the synthesis of mimetics of **5** using the principles of combinatorial chemistry [163-165]. Thioglycosides are also stable to the action of glycosidases. These structures should prove useful as metabolically stable acceptors for assaying GlcNAcT-V.

5.3 Two Diastereospecifically Deuterium-Labeled Trisaccharide Acceptors (5S, 5R) as Probes for the bound Acceptor Conformation

The diastereospecifically labeled trisaccharide acceptors **5S** and **5R** have allowed us to assign the H-6*pro*R and H-6*pro*S signals in ^{1}H NMR spectra unambiguously. The conformational analysis of the trisaccharides, based on the magnitude of the coupling constants $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$, directly provides information about the rotameric distribution about the C5-C6 bond. The results demonstrated that the synthetic trisaccharide acceptor **5** of GlcNAcT-V exists predominantly in two conformers, gg and gt about the αMan(1→6) linkage in the approximate ratio of 3:2 (gg:gt), with a negligibly low

population of the tg conformer. Lindh and Hindsgaul have previously suggested that GlcNAcT-V preferentially recognizes the gg rotamer [91] (Fig. 49). These labeled acceptors (**5S**, **5R**) should be useful in probing a possible conformational change of the acceptor on binding to recombinant GlcNAcT-V. Knowledge of these acceptor conformations will be of value especially in the design of substrate-based inhibitors for this enzyme.

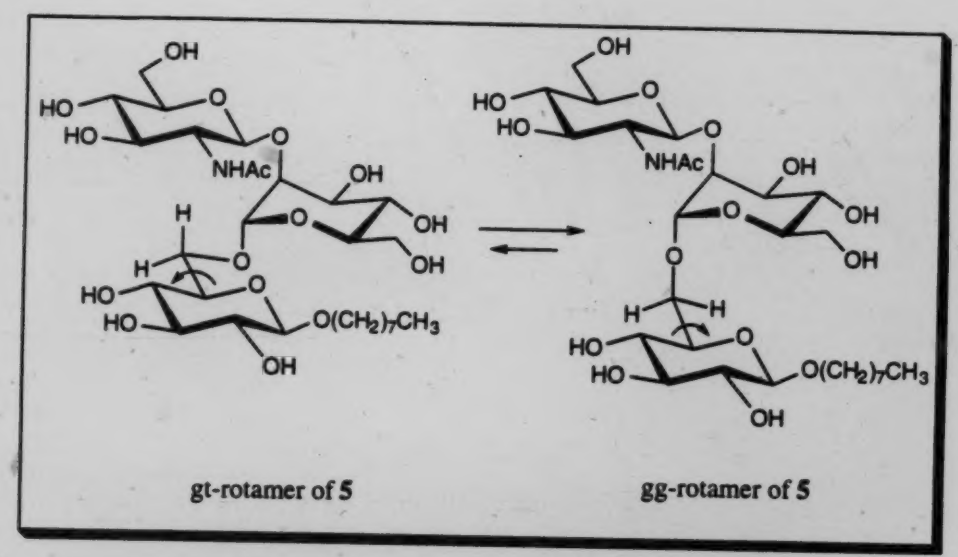


Fig. 49. Rotameric equilibrium about the C5-C6 bond in trisaccharide **5**

5.4 Summary of the Recognition of Synthetic Trisaccharide Analogs by GlcNAcT-V

Fig. 50 summarizes the recognition of synthetic trisaccharide analogs by GlcNAcT-V, based on this thesis research and previous work. For acceptor recognition, only OH-3, OH-4 and OH-6 on the GlcNAc residue are essential. In forming the E-I (or E-S) complexes, both the potentially reactive OH-6 and the neighboring OH-4 on the central

Man residue do not make important contacts with the enzyme. The enzyme further tolerates the replacement of the intersaccharidic linkages of oxygen by sulfur.

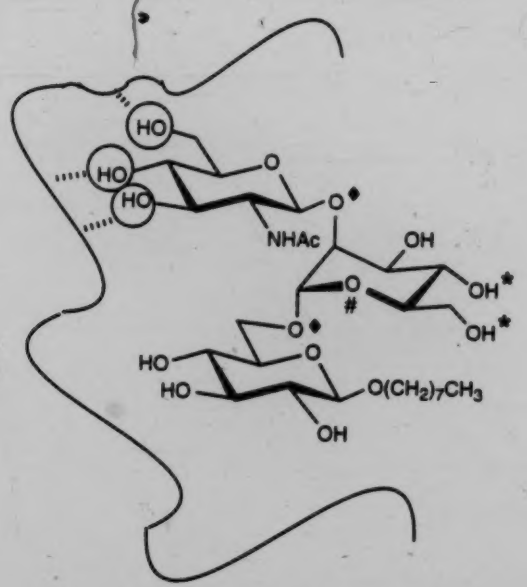


Fig. 50. Summary of GlcNAcT-V recognition of synthetic trisaccharide acceptor analogs. Circled groups could not be replaced by other functional groups. The NHAc group could be replaced by an OH group. Replacement of the hydroxyl groups marked with (*) affects catalysis but not recognition. The oxygen atoms marked with (♦) could be substituted by sulfur. The oxygen atom marked with (#) could be replaced by CH_2. The hydroxyl groups without marks are not essential.

5.5 Future Prospects

The synthesis of trisaccharide inhibitors is extremely time consuming. Therefore, we are now investigating a new approach based on combinatorial chemistry for the synthesis of glycomimmetics as enzyme inhibitors. Combinatorial chemistry is an emerging new and potentially very powerful alternative approach for finding biologically active compounds by synthesizing and testing large chemical libraries containing populations of related molecular structures [163-165]. Since only the terminal Glc (or GlcNAc) residue is essential for

recognition by GlcNAcT-V, and GlcNAcT-V tolerates the substitution of the natural oxygen linkage of acceptor by sulfur linkage, new strategies to create libraries of molecules containing key recognition elements are suggested. A general strategy (Fig. 51) would be to incorporate a Glc-residue as the terminal and essential recognition element to produce glycomimetic inhibitor libraries. Essentially quantitative organic reactions could be used in tandem: Michael addition of thiols, alkylations of thiols, and reductive aminations which can be followed by *N*-alkylation or *N*-acylation. Using commercial Michael acceptors (over 50 available) and acylating agents (over 50 amino acids and hundreds of others), libraries of thousands of compounds can be prepared for enzyme testing [166]. A general view of this strategy is summarized in Fig. 51.

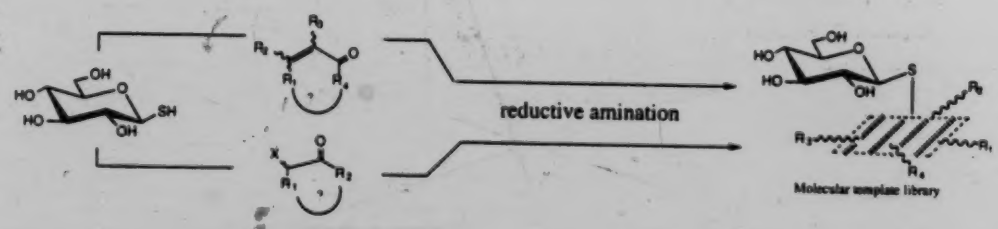


Fig. 51. Application of combinatorial chemistry for the production of glycomimetic libraries

CHAPTER 6

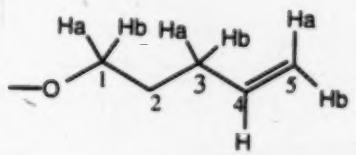
Experimental

General methods:

TLC was performed on Silica gel 60-F254 (E. Merck) with detection by quenching of fluorescence, by charring with H_2SO_4, and/or by reaction with ninhydrin. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (E. Merck, 40-63 μm). Beaded silica gel 6RS-8060 (Iatrobeads) was from Iatron Laboratories, Inc. (Japan). C-18 silica gel (reverse phase) was from Toronto Research Chemicals. C-18 Sep-Pak sample preparation cartridges and Millex-GV (0.22 μm) filter units were from Waters Associates (Mississauga, ON). Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22 °C. IR spectra were recorded with a Nicolet SX-20 FTIR by the spectral services laboratory of the Chemistry Department. ^1H NMR spectra were recorded at 360 MHz (Bruker AMR 360), at 400 MHz (Bruker AM 400), or at 500 MHz (Varian UNITY 500) on solutions in $CDCl_3$ (internal Me_4Si, δ 0), or D_2O. The chemical shifts and coupling constants (as observed splittings) for ^1H NMR are reported as though they were first order. ^{13}C NMR spectra were recorded at 75.5 MHz, 100.6 MHz, or at 125 MHz respectively, on the same instruments in $CDCl_3$ (internal Me_4Si δ 0) or D_2O (internal 1,4-dioxane, δ 67.4). The assignments of ^{13}C NMR signals are tentative. FAB-mass spectra (FAB MS) were obtained on a Kratos AEI-MS9 instrument using Xe as the bombarding gas and glycerol and HCl as the matrix. Electrospray ionization mass spectra (ESI-MS) were obtained from a Micromass ZabSpec Hybrid Sector-TOF instrument using methanol-toluene as liquid carrier. Elemental analyses were carried out on a Carlo Erba EA1108 and all samples submitted for elemental analyses were dried overnight under vacuum over phosphorus pentoxide at 56 °C (reflux acetone). Unless otherwise stated, all reactions were

carried out at room temperature, and in the processing of reaction mixtures, solutions of organic solvents were washed with equal volumes of aqueous solutions. Organic solutions were dried ($MgSO_4$) prior to concentration under vacuum at < 40°C (bath). All anhydrous reactions were carried out under an argon atmosphere.

Protons and carbons of the pentenyl group present in the compounds described in this thesis designated as defined below. In ^{1}H-NMR spectra, pentenyl C_5-Ha (dddd, $J_{5a,4}$ = 17.0 Hz, $J_{5a,5b}$, $J_{5a,3a}$, $J_{5a,3b}$ = 1.5 Hz); pentenyl C_5-H_b (dddd, $J_{5b,4}$ = 10.0 Hz, $J_{5b,3a}$, $J_{5b,3b}$ = 1.5 Hz); pentenyl C_4-H (dddd, $J_{4,3a}$ = 6.6 Hz, $J_{4,3b}$ = 6.5 Hz); pentenyl C_3-H (ddddd, $J_{2,3}$ = 7.0 Hz); pentenyl C_2-H (p, J_{vic} = 7.0 Hz); pentenyl C_1-Ha (ddd, $J_{1a,1b}$ 9.5 Hz, $J_{1a,2}$ = 7.0 Hz); C_1-H_b (ddd, $J_{1b,2}$ = 7.0 Hz).



4-Pentenyl 4,6-O-(4-methoxybenzylidene)-α-D-mannopyranoside (**23**).

4-Pentenyl α-D-mannopyranoside [114] (9.89 g, 39.87 mmol) was dissolved in dry *N*, *N*-dimethylformamide (DMF, 80 mL, 80 °C) and pyridinium *p*-toluenesulfonate (100 mg, 0.4 mmol) was added. 4-Methoxybenzaldehyde dimethyl acetal (8.62 g, 47.36 mmol) in DMF (100 mL) was then added dropwise under a stream of argon so as to remove the liberated methanol. After 24 hrs, TLC indicated complete consumption of starting material. The solvent was evaporated *in vacuo*. The residue was purified by flash chromatography on silica gel, using first hexane-EtOAc (6:1) to remove the side products, and then hexane-EtOAc (1:2) and EtOAc to elute the product. Compound **23** was obtained as a colorless syrup (6.71 g, 46%); $[\alpha]_D$ +49.8° (*c* 1.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360

MHz): δ 7.43 (ddd, 2H, J 8.8, 2.1, 2.0 Hz, PMB C_2-H, C_6-H), 6.90 (ddd, 2H, J 8.8, 2.1, 2.0 Hz, PMB C_3-H, C_5-H), 5.83 (pentenyl C_4-H), 5.56 (s, 1H, PMBCH), 5.05 (pentenyl C_5-H_a), 5.02 (pentenyl C_5-H_b), 4.89 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.27 (dd, 1H, $J_{2,3}$ 3.5 Hz, H-2), 4.15-4.08 (m, 2H), 3.93 (dd, 1H, $J_{3,4}$ 7.5, $J_{4,5}$ 7.5 Hz, H-4), 3.90-3.80 (m, 2H), 3.84 (s, 3H, OCH_3), 3.75 (pentenyl C_1-H_a), 3.47 (pentenyl C_1-H_b), 2.58 (d, 1H, $J_{3,OH}$ 2.2 Hz, C_3-OH), 2.56 (d, 1H, $J_{2,OH}$ 2.9 Hz, C_2-OH), 2.18 (pentenyl C_3-H), 1.74 (pentenyl C_2-H). ^{13}C NMR (75.5 MHz): δ 160.32 (PMB C-4), 137.91 (pentenyl C-4), 129.79 (PMB C-1), 127.62 (PMB C-2), 115.14 (pentenyl C-5), 113.77 (PMB C-3), 102.23 (PMBCH), 100.22 (C-1), 78.96 (C-5), 71.08 (C-2), 68.88 (pentenyl C-1), 68.88 (C-3), 67.29 (C-6), 63.09 (C-4), 55.37 (OCH_3), 30.29, 28.63 (pentenyl C-2/C-3). Anal. Calcd for $C_{19}H_{26}O_7$ (366.41): C, 62.28; H, 7.15. Found: C, 61.93; H, 7.04.

4-Pentenyl 3-O-benzyl-4,6-O-(4-methoxybenzylidene)-α-D-mannopyranoside (**24**).

A suspension of compound **23** (5.90 g, 16.12 mmol) and dibutyltin oxide in methanol (600 mL) was heated at reflux for 3 hrs. Solvent was removed under diminished pressure, leaving a colorless syrup. This residue was dissolved in dry DMF (600 mL), then benzyl bromide (2.90 mL, 24.18 mmol) was added and the resulting solution was brought to 100 °C. After 20 minutes, TLC showed complete disappearance of starting material. The reaction mixture was then concentrated under diminished pressure. Chromatography (2:1 hexane-EtOAc) of the residue afforded **24** (6.32 g, 86%) as a colorless oil; $[\alpha]_D$ +31.5° (*c* 1.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 360 MHz): δ 7.44 (d, 2H, J 8.9 Hz, PMB C_2-H, C_6-H), 7.39-7.28 (m, 7H, Ar-H), 6.89 (d, 2H, J 8.9 Hz, PMB C_3-H, C_5-H), 5.81 (pentenyl C_4-H), 5.59 (s, 1H, PMBCH), 5.05 (pentenyl C_5-H_a), 4.98 (pentenyl C_5-H_b), 4.87 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.85, 4.70(d, each 1H, J_{gem} 12 Hz, $PhCH_2$), 4.25 (dd, 1H, J_{gem} 16.5, $J_{5,6}$ 6.0 Hz, H-6), 4.12-4.05 (m, 2H), 3.93 (dd, $J_{3,4}$ 9.5, $J_{2,3}$ 3.5 Hz, H-3), 3.87-3.78 (m, 3H), 3.71 (pentenyl C_1-H_a), 3.42 (pentenyl

C_1-H_b), 2.65 (d, 1H, $J_{2,OH}$ 1.5 Hz, C_2-OH), 2.12 (pentenyl C_3-H), 1.68 (pentenyl C_2-H); ^{13}C NMR (75.5 MHz): δ 160.05 (PMB C-4), 138.14 (benzyl C-1), 137.92 (pentenyl C-4), 130.15 (PMB C-1), 127.91, 127.83, 127.89 (benzyl methine), 115.09 (pentenyl C-5), 113.61 (PMB C-3), 101.59 (PMBCH), 100.03 (C-1), 78.91 (C-5), 75.83 (C-3), 73.11 benzyl, $PhCH_2$), 70.14 (C-2), 68.90 (pentenyl C-1), 67.21 (C-6), 63.37 (C-2), 55.33 (OCH_3), 30.27, 28.59 (pentenyl C-2/C-3). Anal. Calcd for $C_{26}H_{32}O_7$ (456.54): C, 68.40; H, 7.07. Found: C, 67.92; H, 6.99.

4-Pentenyl 2-O-benzoyl-3-O-benzyl-4,6-O-(4-methoxylbenzylidene)-α-D-mannopyranoside (**25**).

To a solution of **24** (5.92 g, 13.0 mmol) in dry pyridine (50 mL) was added benzoyl chloride (3.84 mL, 33.8 mmol). The reaction mixture was stirred at r.t. overnight. The reaction mixture was poured into ice water (200 mL) and the aqueous solution was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with water, dried over anhydrous $MgSO_4$, and then concentrated. Column chromatography (hexane-EtOAc 6:1) afforded **25** (5.92 g, 81%) as a colorless oil; $[\alpha]_D$ -35.9° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.11 (dd, 2H, J 8.5, 1.5 Hz, benzoyl, C_2-H, C_6-H), 7.59 (dddd, 1H, J 8.0, 7.9, 1.5, 1.5 Hz, benzoyl, C_4-H), 7.50-7.22 (m, 9H, Ar-H), 6.90 (d, 2H, J 8.5 Hz, PMB C_3-H, C_5-H), 5.82 (pentenyl C_4-H), 5.67 (s, 1H, PMBCH), 5.61 (dd, 1H, $J_{2,3}$ 2.9, $J_{1,2}$ 1.5 Hz, C_2-H), 5.07 (pentenyl C_5-H_a), 5.02 (pentenyl C_5-H_b), 4.95 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.78, 4.72 (d, each 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.29 (dd, 1H, $J_{3,4}$ 9.2 Hz, H-3), 4.21-4.12 (m, 2H), 3.99-3.85 (m, 2H), 3.82 (s, 3H, OMe), 3.75 (pentenyl C_1-Ha), 3.47 (pentenyl C_1-Hb), 2.17 (pentenyl C_3-H), 1.75 (pentenyl C_2-H); ^{13}C NMR (75.5 MHz): δ 165.83 (C=O), 160.03 (PMB C-4), 138.13 (benzyl, C-1), 137.82 (pentenyl C-4), 133.28 (benzoyl, C-4), 130.04 (PMB C-1), 129.94 (benzoyl C-4), 129.84 (benzoyl C-2, C-5), 128.43, 128.26, 122.58, 122.50, 122.44 (aromatic methine), 115.17 (pentenyl C-5), 113.54 (PMB C-3), 101.64 (PMBCH), 98.82 (C-1), 78.74 (C-5), 74.12

(C-3), 72.02 ($PhCH_2$), 70.46 (C-2), 68.87 (pentenyl C-1), 67.49 (C-6), 63.97 (C-4), 55.29 (OMe), 30.23, 28.54 (pentenyl C-2/C-3). Anal. Calcd for $C_{33}H_{36}O_8$ (560.65): C, 70.70; H, 6.47. Found: C, 70.62; H, 6.39.

4-Pentenyl 2-O-benzoyl-3-O-benzyl-6-(4-methoxylbenzyl)-α-D-mannopyranoside (**26**).

A mixture of compound **25** (5.36 g, 9.56 mmol), sodium cyanoborohydride (6.01 g, 95.7 mmol), crushed molecular sieves 3 Å (10 g) and dry DMF (80 mL) was cooled to 0 °C. Trifluoroacetic acid (7.33 mL, 95.7 mmol) in DMF (60 mL) was then added dropwise. The mixture was stirred at 0° C for 24 hrs, then allowed to warm to r.t. and stirred for another 24 hrs. After filtration of the reaction mixture through Celite, the filtrate was diluted with CH_2Cl_2 and washed with a cold saturated aqueous solution of sodium bicarbonate and ice water. The organic layer was dried ($MgSO_4$), then concentrated. Chromatography of the residue on silica gel (4:1 hexane-EtOAc) afforded compound **26** (4.55 g, 85%) as a colorless oil; $[\alpha]_D$ -15.1° (*c* 2.0, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 8.05 (dd, 2H, J 8.5, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.55 (dddd, 1H, J 8.0, 7.9, 1.5, 1.5 Hz, benzoyl C_4-H), 7.40 (dd, 2H, J 8.5, 7.9 Hz, benzoyl C_3-H, C_5-H), 7.32-7.23 (m, 7H, Ar-H), 6.87 (d, 2H, J 8.5 Hz, PMB C_3-H, C_5-H), 5.82 (pentenyl C_4-H), 5.59 (d, 1H, $J_{2,3}$ 3.2 Hz, $J_{1,2}$ 2.0 Hz, H-2), 5.07 (pentenyl C_5-H_a), 5.02 (pentenyl C_5-H_b), 4.98 (d, 1H, H-1), 4.79 (d, 1H, J_{gem} 11.3 Hz, $PMBCH_2$), 4.62 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.52 (d, 1H, J_{gem} 11.3 Hz, $PMBCH_2$), 4.51 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.13 (dd, 1H, $J_{4,5}$ 10.0, $J_{3,4}$ 9.5 Hz, H-4), 3.91 (dd, 1H, H-3), 3.89-3.72 (pentenyl C_1-Ha), 3.82 (s, 3H, OMe), 3.50 (pentenyl C_1-Hb), 2.56 (d, 1H, J 2.0 Hz, C_4-OH), 2.15 (pentenyl C_3-H), 1.74 (pentenyl C_2-H); ^{13}C NMR (75.5 MHz): δ 165.82 (C=O), 159.16 (PMB C-4), 137.96 (pentenyl C-4), 137.75 (benzyl C-1), 133.18 (benzoyl C-4), 129.94, 129.12, 128.46, 128.40, 128.10, 127.88 (Aromatic CH), 115.05 (pentenyl C-5), 113.77 (PMB C-3), 98.00 (C-1), 77.72 (C-5), 73.32 ($PMBCH_2$), 71.47 ($PhCH_2$), 71.42 (C-3), 69.73 (pentenyl C-1), 68.55 (C-2), 67.55 (C-4), 67.37 (C-6), 55.29 (OMe), 30.30, 28.62

(pentenyl C-2/C-3). Anal. Calcd for $C_{33}H_{38}O_8$ (562.67): C, 70.44; H, 6.81. Found: C, 70.41; H, 6.82.

4-Pentenyl 2-O-benzoyl-3-O-benzyl-6-(4-methoxylbenzyl)-4-O-methyl-α-D-mannopyranoside (**27**).

To a solution of compound **26** (4.36 g, 7.75 mmol) in dry DMF (110 mL) at -18 °C, methyl iodide (730 µL, 11.731 mmol) was added dropwise, then sodium hydride (60% dispersion in oil, 626 mg, 15.64 mmol) was added. The reaction mixture was stirred at -15 °C for 3 hrs. Acetic acid was added to quench the reaction (until pH < 7). The reaction mixture was concentrated *in vacuo* and the residue was extracted with CH_2Cl_2. After solvent evaporation, the residue was purified by chromatography (hexane-EtOAc 6:1), yielding **27** (4.04 g, 90%) as a colorless oil; $[\alpha]_D$ -16.7° (*c* 1.3, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 8.07 (dd, 2H, J 8.5, 1.5 Hz, benzoyl, C_2-H), 7.56 (ddd, 1H, J 8.0, 7.9, 1.5, 1.5 Hz, benzoyl, C_4-H), 7.42-7.20 (m, 9H, Ar-H), 6.89 (d, 2H, J 8.5 Hz, PMB, C_3-H, C_5-H), 5.83 (pentenyl C_4-H), 5.58 (dd, 1H, $J_{2,3}$ 3.1, $J_{1,2}$ 2.0 Hz, H-2), 5.07 (pentenyl C_5-H_a), 5.02 (pentenyl C_5-H_b), 4.96 (d, 1H, H-1), 4.80, 4.54 (d, each 1H, J_{gem} 11.3 Hz, $PMBCH_2$), 4.70, 4.60 (d, each 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.00 (dd, 1H, $J_{3,4}$ 9.5 Hz, H-3), 3.90-3.70 (m, 5H, H-4, H-5, H-6, pentenyl C1-H_a), 3.82 (s, 3H, PMB OMe), 3.57 (s, 3H, C_4-OMe), 3.48 (pentenyl C_1-H_b), 2.15 (pentenyl C_3-H), 1.70 (pentenyl C_2-H); ^{13}C NMR (75.5 MHz): δ 165.83 (C=O), 159.13 (PMB C-4), 138.24 (benzyl C-1), 137.99 (pentenyl C-4), 133.07 (benzoyl C-4), 130.74 (PMB C-1), 130.03 (benzoyl C-1), 129.99, 129.12, 128.38, 128.88, 127.55 (Aromatic CH), 78.14 (C-5), 76.19 (C-4), 73.18 ($PMBCH_2$), 71.73 (C-3), 71.56 ($PhCH_2$), 69.26 (C-2), 68.98 (pentenyl C-1), 67.29 (C-6), 61.02 (C_4-OMe), 55.33 (PMB-OMe), 30.30, 28.64 (pentenyl C-2/C-3); Anal. Calcd for $C_{34}H_{40}O_8$ (576.69): C, 70.81; H, 6.99. Found: C, 71.11; H, 7.07.

4-Pentenyl 2-O-benzoyl-3-O-benzyl-4-O-methyl-α-D-mannopyranoside (**28**).

To a solution of compound **27** (3.96 g, 6.86 mmol) in CH_2Cl_2-H_2O (19:1, 150 mL) was added a solution of DDQ (1.87 g, 8.24 mmol) in CH_2Cl_2-H_2O (19:1). The resulting mixture was stirred at r.t. under argon for 5 hrs. The reaction mixture was filtered through Celite and the filtrate was concentrated to dryness. The residue was then purified by silica gel flash chromatography, eluting first with CH_2Cl_2 to remove the anisaldehyde, then with CH_2Cl_2-MeOH (99:1) to yield the desired product (2.72 g, yield 87%) as a colorless oil; $[\alpha]_D$ -0.80° (*c* 1.9, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$), δ 8.05 (dd, 2H, J 8.5, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.60 (dddd, 1H, J 8.0, 7.9, 1.5, 1.5 Hz, benzoyl C_4-H), 7.48 (dd, 2H, J 8.5, 8.0 Hz, benzoyl, C_3-H, C_5-H), 7.30 (m, 5H, benzyl Ar-H), 5.82 (pentenyl C_4-H), 5.55 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.2 Hz, H-2),5.05 (pentenyl C_5-H_a), 5.01 (pentenyl C_5-H_b), 4.90 (d, 1H, H-1), 4.78, 4.60(d, each 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.00 (dd, 1H, $J_{3,4}$ 8.5 Hz, H-3), 3.90-3.80 (m, 2H, H-5, H-6), 3.75-3.65 (m, 3H), 3.45 (pentenyl C_1-Ha), 3.61 (s, 3H, OMe), 2.14 (pentenyl C_3-H), 2.04 (dd, 1H, $J_{6a,OH}$ 8.1, $J_{6b,OH}$ 5.5 Hz, C_6-OH), 1.71 (C_2-H); ^{13}C NMR (75.5MHz): δ 165.78 (C=O), 138.14 (benzyl C-1), 137.89 (pentenyl C-4), 133.27 (benzoyl C-4), 129.89, 128.50, 128.30, 127.84, 127.59 (Aromatic CH), 115.17 (pentenyl C-5), 97.86 (C-1), 77.94 (C-5), 78.28 (C-4), 71.87(C-3), 71.59 ($PhCH_2$), 69.34 (C-2), 67.36 (pentenyl C-1), 62.20 (C-6), 61.15 (C_4-OMe), 30.25, 28.59 (pentenyl C-2/C-3); Anal. Calcd for $C_{26}H_{32}O_7$ (456.54): C, 68.40; H, 7.07. Found: C, 68.37; H, 7.06.

4-Pentenyl 2-O-benzoyl-3-O-benzyl-4-O-methyl-6-O-tosyl-α-D-mannopyranoside (**29**).

To a solution of **28** (1.32 g, 2.89 mmol) in dry pyridine (60 mL), *p*-toluenesulfonyl chloride (5.52 g, 28.9 mmol) was added. The reaction mixture was stirred at r.t. for 7 hrs, then poured into cold 5% $NaHCO_3$ (200 mL) and the aqueous solution was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with 0.5% aq. HCl and water, then dried ($MgSO_4$). Concentration gave **29** (1.77 g, quant.) as a light yellowish oil. ^1H NMR

($CDCl_3$): δ 8.05 (dd, 2H, J 8.5, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.87 (d, 2H, J 8.0 Hz, tosyl C_2-H, C_6-H), 7.59 (dddd, 1H, J 8.0, 7.9, 1.5, 1.5 Hz, benzoyl C_4-H), 7.48 (dd, 2H, J 8.5, 8.0 Hz, benzoyl C_3-H, C_5-H), 7.28 (m, 8H, Ar-H), 5.79 (pentenyl C_4-H), 5.50 (dd, 1H, $J_{1,2}$ 1.9, $J_{2,3}$ 3.2 Hz, H-2), 5.05 (pentenyl C_5-H_a), 4.98 (C_5-H_b), 4.88 (d, 1H, H-1), 4.75, 4.55 (d, each 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.39 (dd, 1H, J_{gem} 10.5, $J_{5,6}$ 4.0 Hz, H-6a), 4.28 (dd, J_{gem} 10.5, $J_{5,6b}$ 1.9 Hz, H-6b), 3.95 (dd, 1H, $J_{3,4}$ 8.5 Hz, H-3), 3.75 (ddd, 1H, $J_{4,5}$ 9.5, $J_{5,6}$ 4.0, 1.9 Hz, H-5), 3.62 (pentenyl C_1-H_a), 3.61 (dd, 1H, H-4), 3.51 (s, 3H, OMe), 3.38 (pentenyl C_1-H_b), 2.09 (pentenyl C_3-H), 1.68 (pentenyl C_2-H).

4-Pentenyl 6-azido-2-O-benzoyl-3-O-benzyl-6-deoxy-4-O-methyl-α-D-mannopyranoside (**15**).

To a solution of **29** (1.77 g, 2.89 mmol) in dry DMF (50 mL) was added sodium azide (1.88 g, 28.9 mmol). The resulting mixture was stirred at r.t. for 12 hrs, then at 40 °C for 20 hrs. Concentration left a residue which was extracted with CH_2Cl_2, then washed with water, dried ($MgSO_4$) and concentrated. The residue was purified by chromatography, eluting with hexane-ethyl acetate (9:1) to give **15** (1.26 g, yield 90%) as a colorless oil; $[\alpha]_D$ +53.1° (*c* 0.2, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 8.08 (dd, 2H, J 8.5, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.59 (dddd, J 8.0, 7.9, 1.5, 1.5 Hz, benzoyl C_4-H), 7.47 (dddd, 2H, J 8.5, 8.0, 1.5, 1.5 Hz, benzoyl C_3-H, C_5-H), 7.35-7.21 (m, 5H, Ar-H), 5.82 (pentenyl C_4-H), 5.58 (dd, 1H, $J_{1,2}$ 1.9, $J_{2,3}$ 3.2 Hz, H-2), 5.05 (pentenyl C_5-H_a), 5.00 (pentenyl C_5-H_b), 4.89 (d, 1H, H-1), 4.78, 4.57 (d, each 1H, J_{gem} 11.5 Hz, $PhCH_2$), 3.98 (dd, 1H, $J_{3,4}$ 8.8 Hz, H-3), 3.78 (ddd, 1H, $J_{4,5}$ 9.5, $J_{5,6b}$ 5.5, $J_{5,6a}$ 2.8 Hz, H-5), 3.74 (pentenyl C_1-H_a), 3.59 (dd, 1H, H-4), 3.60 (dd, J_{gem} 13.0 Hz, H-6_a), 3.58 (s, 3H, OMe), 3.49 (pentenyl C_1-H_b), 3.47 (dd, 1H, J_{gem} 13.0 Hz, H-6_b), 2.15 (pentenyl C_3-H), 1.75 (pentenyl C_2-H); ^{13}C NMR (75.5 MHz): δ 165.78 (C=O), 138.82 (benzyl C-1), 137.89 (pentenyl C-4), 133.25 (benzyl C-4),

129.94, 128.47, 128.32, 127.91, 127.64 (Aromatic CH), 115.12 (pentenyl C-5), 97.80 (C-1), 77.92 (C-5), 76.90 (C-4), 71.54 ($PhCH_2$), 71.46 (C-3), 69.04 (C-2), 67.51 (pentenyl C-1), 61.18 (OMe), 51.53 (C-6), 30.27, 28.59 (pentenyl C-2/C-3); IR ($CHCl_3$): 2099 cm^{-1} (N_3), 1725 cm^{-1} (ester); Anal. Calcd for $C_{26}H_{31}N_3O_6$ (481.55): C, 64.85; H, 6.44; N, 8.73. Found: C, 64.67; H, 6.58; N, 8.66.

Octyl 6-azido-2-O-benzoyl-3-O-benzyl-6-deoxy-4-O-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**30**).

A solution of **15** (482 mg, 1.00 mmol) and octyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside [88] (620 mg, 1.10 mmol) in toluene was co-evaporated to dryness in *vacuo*. The dried residue was dissolved in anhydrous dichloromethane and was cooled to 0° C. *N*-Iodosuccinimide (270 mg, 1.20 mmol) and AgOTf (6.0 mg, 22.5 μmol) were added to the solution. After stirring at r.t. for 20 hrs, the reaction mixture was diluted with CH_2Cl_2, and then washed sequentially with 10% $Na_2S_2O_3$, then saturated sodium bicarbonate and finally brine. The organic phase was dried ($MgSO_4$), filtered and concentrated. The residue was purified by chromatography using hexane-ethyl acetate (9:1) as eluant to provide the title compound (**30**) as a colorless oil (455 mg, 48%); $[\alpha]_D$ +39.3° (*c* 0.4, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$): δ 8.08 (dd, 2H, J 8.5, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.59 (dddd, 1H, J 8.0, 7.9, 1.5, 1.5 Hz, benzoyl C_4-H), 7.47 (dddd, 2H, J 8.5, 8.0, 1.5, 1.5 Hz, benzoyl C_3-H, C_5-H), 7.38-7.18 (m, 20H, benzyl Ar-H), 5.65 (dd, 1H, $J_{1',2'}$ 1.9 Hz, $J_{2',3'}$ 3.2 Hz, H-2'), 4.97 (d, 1H, J_{gem} 9.5 Hz, $PhCH_2$), 4.96 (d, 2H, J_{gem} 10.5 Hz, $PhCH_2$), 4.88 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.78 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.75 (d, 1H, H-1'), 4.73 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.55 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.49 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.38 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 3.92 (dd, 1H, $J_{3',4'}$ 8.8 Hz, H-3'), 3.36 (dd, 1H, J_{gem} 13.0, $J_{5',6'}$ 5.0 Hz, H-6'), 3.52 (s, 3H, OMe), 1.62 (p, 2H, J_{vic} 7.0 Hz, octyl CH_2), 0.84 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 165.59 (benzoyl C=O), 138.62 (benzoyl,

C-1), 138.56, 138.07, 137.97 (benzyl C-1), 133.21 (benzoyl C-4), 129.97 (benzoyl C-2, C-6), 128.48, 128.42, 128.33, 128.19, 128.00, 127.94, 127.80, 127.71, 127.66 (Aromatic CH), 103.61 ($J_{C-1,H-1}$ 161.5 Hz, C-1), 97.93 ($J_{C-1',H-1'}$ 170.9 Hz, C-1'), 70.17 (C-6), 66.57 (octyl C-1), 61.06 (OCH_3), 51.37 (C-6'), 31.88, 29.85, 29.48, 29.31, 26.21, 22.71 (octyl C_2-C_6), 14.13 (octyl CH_3); Anal. Calcd for $C_{56}H_{67}O_{11}N_3$(958.17): C, 70.20; H, 7.05; N, 4.39. Found: C, 70.30; H, 6.90; N, 4.39.

Octyl O-(6-azido-3-O-benzyl-6-deoxy-4-O-methyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**31**).

Compound **30** (427 mg, 0.446 mmol) was treated with methanolic NaOMe (0.05 N, 25 mL) at r.t. for 36 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration and solvent evaporation left a residue which was purified by column chromatography using hexane-EtOAc (4:1) as eluant to provide compound **31** (white amorphous powder, 365 mg, 96%). ^{1}H NMR (360 MHz, $CDCl_3$), δ 7.40-7.22 (m, 20H, Ar-H), 4.98 (d, 1H, J_{gem} 10.4 Hz, $PhCH_2$), 4.97 (d, 2H, J_{gem} 11.0 Hz, $PhCH_2$), 4.80 (d, 1H, J_{gem} 10.6 Hz, $PhCH_2$), 4.75 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.73 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.71 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.56 (d, 1H, J_{gem} 10.6 Hz, $PhCH_2$), 4.38 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.08 (ddd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.51 (s, 3H, OMe), 2.35 (d, 1H, $J_{2',OH}$ 2.0 Hz, $C_{2'}$-OH), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl OCH_3).

Octyl O-(2-deoxy-2-phthalimido-3,4,6-tri-O-acetyl-β-D-glucopyranosyl)-(1→2)-O-(6-azido-3-O-benzyl-6-deoxy-4-O-methyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**32**).

A mixture of **31** (136 mg, 0.159 mmol), AgOTf (102 mg, 0.398 mmol), molecular sieves 4 Å (350 mg), collidine (19.0 μL, 0.143 mmol) and dry CH_2Cl_2 (2 mL) was cooled to -40 °C. A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-glucopyranosyl bromide

(**14**) [120] (159 mg, 0.318 mmol) in dry dichloromethane (1 mL) was then added dropwise at -40 ^{o}C under argon. The mixture was allowed to warm to room temperature within 1 h. After stirring for 3 hrs, excess tetraethylammonium chloride (67 mg, 0.40 mmol) was added, and the mixture was stirred for another 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through celite. The filtrate was washed sequentially with 0.5% HCl, sat. $NaHCO_3$ and water, and then dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography using hexane-EtOAc (2:1) as eluant to provide compound **32** as a white crystal (148 mg, 73%). $[\alpha]_D$ +13.2^{o} (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, 2H, J 5.1, 2.9 Hz, phthalimido Ar-H), 7.71 (dd, 2H, J 5.1, 2.9 Hz, phthalimido Ar-H), 7.39-7.12 (m, 20H, Ar-H), 5.83 (dd, 1H, $J_{2",3"}$ 10.5 Hz, $J_{3",4"}$ 9.5 Hz, H-3"), 5.48 (d, 1H, $J_{1",2"}$ 8.3 Hz, H-1"), 5.20 (dd, $J_{4",5"}$ 9.8 Hz, H-4"), 4.98 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.95 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.77 (2d, each 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.77 (2d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.72 (2d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.64 (d, 1H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.50 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.46 (dd, 1H, J_{gem} 12.8 Hz, $J_{6",5"}$ 2.8 Hz, H-6"), 4.45 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.44 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.75 (dd, 1H, $J_{3',4'}$ 11.0, $J_{2',3'}$ 3.8 Hz, H-3'), 3.34 (s, 3H, OMe), 2.06, 2.04, 1.89 (3s, each 3H, $\underline{CH_3}CO$), 0.88 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 170.71, 170.15, 169.45 (C=O), 138.58, 138.45, 138.05, 137.96 (benzyl C-1), 103.92 (C-1), 97.51 (C-1'), 96.24 (C-1"), 70.73 (octyl C-1), 66.75 (C-6), 62.39 (C-6"), 60.85 (OCH_3), 54.38 (C-2"), 51.41 (C-6'), 31.89, 29.86, 29.54, 29.33, 26.23, 22.70 (octyl C_2-C_6), 20.80, 20.67, 20.53 ($\underline{CH_3}CO$), 14.12 (octyl CH_3); Anal. Calcd for $C_{69}H_{82}O_{19}N_4$ (1271.44): C, 65.18; H, 6.50; N, 4.41. Found: C, 65.19; H, 6.48; N, 4.40.

Octyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-(6-azido-3-O-benzyl-6-deoxy-4-O-methyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**33**).

A solution of **32** (140 mg, 0.110 mmol) in *n*-butanol (20 mL) was added to ethylenediamine (1 mL). The reaction mixture was stirred at 70 °C for 14 hrs. Concentration of the mixture was followed by two successive additions and evaporations twice of toluene, then one of methanol. The residue was not characterized but was dissolved in dry methanol (3 mL) to which acetic anhydride (1 mL) and triethylamine (0.1 mL) were added. After stirring for 12 hrs at r.t., water (1 mL) was added, and the solution was concentrated. The residue was purified on Iatrobeads using CH_2Cl_2-MeOH (10:1) as eluant, to give **33** as a colourless syrup (85 mg, 73%). $[\alpha]_D$ +6.1° (*c* 0.15, $CHCl_3$); ^1H NMR ($CDCl_3$, 360 MHz): δ 7.42-7.16 (m, 20H, Ar-H), 6.28 (br s, 1H, NHAc), 4.96 (2d, each 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.87 (2d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.79 (2d, each 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.72 (d, 1H, $J_{1',2'}$ 2.0 Hz, H-1'), 4.63 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.55 (d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1''), 4.43 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.36 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.12 (dd, 1H, $J_{2',3'}$ 3.5 Hz, H-2'), 3.53 (s, 3H, OMe), 1.95 (s, 3H, NHAc), 0.84 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 138.49, 138.39, 138.09, 137.64 (benzyl C-1), 128.61, 128.45, 128.31, 127.99, 127.86, 127.77, 127.68 (Aromatic CH), 103.90 (C-1), 99.29 (C-1''), 98.03 (C-1'), 75.82, 74.95, 74.89, 71.92 (PhCH2), 70.74 (octyl C_1), 66.49 (C-6), 62.52 (C-6''), 60.85 (CH_3), 58.82 (C-2''), 51.48 (C-6'), 31.87, 29.82, 29.48, 29.30, 26.18, 22.20 (octyl C_2-C_7), 23.64 ($COCH_3$), 14.13 (octyl CH_3); Anal. Calcd for $C_{57}H_{76}O_{15}N_4$ (1057.26): C, 64.76; H, 7.25; N, 5.30. Found: C, 64.41; H, 7.45; N, 5.16.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-amino-6-deoxy-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11a**).

Compound **33** (98 mg, 92.7 μmol) was dissolved in 95% EtOH (40 mL) containing 5% palladium on charcoal (300 mg), then 0.01N HCl (14 mL) was added. The mixture was stirred under one atmosphere H_2 for 56 hrs. Removal of the catalyst by filtration followed by evaporation left a glass which showed a single spot on TLC. This material was adsorbed onto a Sep-Pak C-18 cartridge in water, the cartridge was washed with water (25 mL) and eluted with HPLC grade methanol (20 mL). Evaporation of the solvent, filtration through a Millex filter and lyophilization of the residue from water gave **11a** (56.6 mg, 91%) as a white powder; $[\alpha]_D$ -7.2° (*c* 0.7, H_2O); ^1H NMR (D_2O, 500 MHz): δ 4.894 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.557 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.451 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.099 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.922 (dd, 1H, $J_{3',4'}$ 8.5 Hz, H-3'), 3.867 (ddd, 1H, J_{gem} 10.0 Hz, J_{vic} 7.0 Hz, octyl C_1-H_a), 3.704 (dd, 1H, $J_{2'',3''}$ 9.6 Hz, H-2''), 3.686 (dd, 1H, $J_{3'',4''}$ 9.6, $J_{4'',5''}$ 9.6 Hz, H-4''), 3.678 (ddd, 1H, octyl C_1-H_b), 3.592 (ddd, 1H, $J_{5'',6''a}$ 5.6, $J_{5'',6''b}$ 2.5 Hz, H-5''), 3.548 (dd, 1H, $J_{2'',3''}$ 9.6 Hz, H-3''), 3.525 (s, 3H, OCH_3), 3.477 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 8.6 Hz, H-3), 3.430 (dd, 1H, J_{gem} 11.0, $J_{5,6'a}$ 3.0 Hz, H-6'a), 3.279 (dd, 1H, $J_{4',5'}$ 9.6 Hz, H-4'), 3.240 (dd, 1H, $J_{2,3}$ 8.0 Hz, H-2), 3.080 (dd, 1H, J_{gem} 11.0, $J_{5',6'b}$ 8.6 Hz, H-6'b), 2.050 (s, 3H, $COCH_3$), 1.615 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 1.38-1.24 (m 12H, octyl CH_2), 0.860 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 175.43 (C=O), 103.23 (C-1, $J_{C\text{-}1,H\text{-}1}$ 161.7 Hz), 100.93 (C-1'', $J_{C\text{-}1'',H\text{-}1''}$ 161.6 Hz), 97.98 (C-1, $J_{C\text{-}1',H\text{-}1'}$ 172.4 Hz), 79.32 (C-4'), 77.91 (C-2'), 76.76 (C-3), 76.66 (C-5''), 74.88 (C-5), 74.16 (C-3''), 73.97 (C-2), 71.82 (octyl C1), 70.83 (C-4), 70.43 (C-3'), 70.23 (C-4''), 68.82 (C-5'), 66.99 (C-6), 61.39 (C-6''), 61.29 (OCH_3), 56.27 (C-2''), 41.70 (C-6'), 31.93, 29.62, 29.27, 29.22, 25.87, 22.86 (octyl C_2-C_7), 23.18 ($\underline{CH_3}CO$), 14.28 (octyl CH_3); FAB MS: m/z 693 $(M+Na)^+$, 671 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-acetamido-6-deoxy-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11b**).

To a solution of **11a** (1.4 mg, 1.96 μmol) in dry methanol (0.5 mL) was added acetic anhydride (0.5 mL) at room temperature. The reaction mixture was stirred for 14 hrs, solvents were removed and the resulting residue was purified as described for the preparation of **11a** to give **11b** (1.5 mg, 100%). ^{1}H NMR (D_2O, 400 MHz): δ 4.83 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.54 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.45 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.07 (dd, 1H, $J_{2',3'}$ 2.3 Hz, H-2'), 3.51 (s, 3H, OCH_3), 3.26 (dd, 1H, $J_{4',5'}$, $J_{3',4'}$ 9.6 Hz, H-4'), 3.24 (dd, 1H, $J_{2,3}$ 8.0 Hz, H-2), 2.06, 2.04 (s, each 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 735 $(M+Na)^+$, 713 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-benzamido-6-deoxy-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11c**).

To a solution of compound **11a** (1.3 mg, 1.94 μmol) in dry pyridine (0.5 mL) was added benzoyl chloride (4.5 μL, 38.8 μmol). The reaction mixture was stirred at r.t. for 24 hrs, and poured into ice water. The aqueous solution was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with 1% HCl, saturated $NaHCO_3$ and water, and then dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography using CH_2Cl_2-MeOH (20:1) as eluant, to provide the perbenzoylated product, which was then treated with 0.05 N NaOMe/MeOH for 24 hrs. Neutralization with Amberlite IR-120 (H^+) resin, filtration of the resin, and evaporation left a residue which was purified as described for the preparation of **11a** to give **11c** (1.0 mg, 62%). ^{1}H NMR (D_2O, 360 MHz): δ 7.78 (dd, 2H, J 8.0, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.65 (ddd, 1H, J 8.0, 7.9, 1.5 Hz, benzoyl C_4-H), 7.57 (dd, 2H, J 8.0, 7.9 Hz, benzoyl C_3-H, C_5-H), 4.89 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.59 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.40 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.11 (dd, $J_{2',3'}$ 3.2 Hz, H-2'), 3.60 (s, 3H, OCH_3), 3.17 (dd, 1H,

$J_{1,2}$, $J_{2,3}$ 8.0 Hz, H-2), 2.08 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 797.6 $(M+Na)^+$, 775.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-4-O-methyl-6-succinamido-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11d**).

A mixture of **11a** (1.1 mg, 1.65 µmol) and succinic anhydride (4.4 mg, 43.8 µmol) in dry methanol (0.5 mL) was stirred at r.t. for 3 hrs and then concentrated to dryness. The product was purified by chromatography on Iatrobeads, eluting first with CH_2Cl_2-MeOH (9:1) to remove excess succinic anhydride, then with $CHCl_3$-MeOH-H_2O (65:35:8) to get the product. The product thus obtained was purified as described for the preparation of **11a** to provide the title compound (1.0 mg, 79%). ^1H NMR (D_2O, 360 MHz): δ 4.88 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.56 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.45 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.08 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.55 (s, 3H, OCH_3), 2.65 (m, 4H, $NHCOCH_2CH_2COOH$), 2.08 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 793.6 $(M+Na)^+$, 771.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy,β-D-glucopyranosyl-(1→2)-6-deoxy-6-iodoacetamido-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11e**).

A mixture of compound **11a** (8.1 mg, 12.1 µmol) and iodoacetic anhydride (73 mg, 206 µmol) in dry methanol (1 mL) was stirred at r.t. for 24 hrs and then concentrated. The residue was dissolved in milli-Q water (1 mL) and was purified as described for the preparation of **11a**. Lyophilization afforded desired product (6.3 mg. 62%). ^1H NMR (D_2O, 400 MHz): δ 4.81 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.54 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.44 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.06 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.52 (s, 3H, OCH_3), 3.28 (dd, 1H, $J_{4',5'}$, $J_{3',4'}$ 9.7 Hz, H-4'), 3.25 (dd, 1H, $J_{2,3}$ 8.3 Hz, H-2), 2.08 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100 MHz): δ 175.73 ($NHCOCH_3$), 172.69 ($COCH_2I$), 103.10 (C-1), 101.17 (C-1''), 97.63 (C-1'),

79.11, 78.16, 76.63, 75.03, 74.18, 73.92, 71.70 (octyl C_1), 70.64, 70.51, 70.44, 67.00 (C-6), 61.44 (C-6"), 61.36 (OCH_3), 56.29 (C-2"), 41.57 (C-6'), 31.93, 29.65, 29.30, 29.24, 25.93, 22.87 (octyl CH_2), 23.33 ($NHCOCH_3$), 14.26 (octyl CH_3), -1.82 (ICH_2); FAB MS: m/z 839.4 $(M+H)^+$, 712.6 $(M-I+H)^+$, 711.6 $(M-I)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-acrylamido-6-deoxy-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11f**).

To a solution of **11a** (1.2 mg, 1.8 μmol) in a mixture of saturated $NaHCO_3$ and 95% EtOH (1:1, 1 mL, $NaHCO_3$ precipitate removed) was added acryloyl chloride (1.4 μL, 18 μmol). The mixture was stirred at r.t. for 2 hrs and concentrated. The residue was purified as described for the preparation of **11a** to afford **11f** (1.3 mg, 99%). ^1H NMR (D_2O, 360 MHz): δ 6.40 (dd, 1H, J_{trans} 17.0, J_{cis} 10.5 Hz, $COCH=CH_2$), 6.23 (d, 1H, J 17.0 Hz, acryloyl C_3-H_a), 5.80 (d, 1H, J 10.5 Hz, acryloyl C_3-H_b), 4.85 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.57 (d, 1H, $J_{1",2"}$ 8.4 Hz, H-1"), 4.45 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.08 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.55 (s, 3H, OCH_3), 3.31 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.7 Hz, H-4'), 3.26 (dd, 1H, $J_{2,3}$ 8.1 Hz, H-2), 2.09 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3), FAB MS: m/z 747.4 $(M+Na)^+$, 725.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-4-O-methyl-6-thiophenylacetamido-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11g**).

To a solution of **11e** (0.78 mg, 0.93 μmol) in 5% $NaHCO_3$ (0.5 mL), a solution of thiophenol (0.19 μL, 1.86 μmol) in 95% EtOH (0.1 mL) was added. The mixture was stirred at r.t. for 1 h and then taken to dryness. The residue was purified as described for the preparation of **11a** to give **11g** (0.6 mg, 79%). ^1H NMR (D_2O, 360 MHz): δ 7.48-7.30 (m, 5H, Ar-H), 4.79 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.52 (d, 1H, $J_{1',2'}$ 8.4 Hz, H-1'), 4.41 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.05 (d, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.45 (s, 3H, OCH_3), 3.25 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.7 Hz, H-4'), 3.22 (dd, 1H, $J_{2,3}$ 8.1 Hz, H-2),

2.04 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 843.5 $(M+Na)^+$, 821.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-6-(dimethyl ethylaminoacetamido)-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside Iodide (**11h**).

To a solution of compound **11e** (0.68 mg, 0.81 μmol) in dry methanol (0.5 mL) was added *N,N*-dimethylethylamine (0.2 mL). The reaction mixture was stirred at r.t. for 1 h and then taken to dryness. The residue was dissolved in water and lyophilized to give **11h** (0.6 mg, 81%) as a white powder. ^1H NMR (D_2O, 360 MHz): δ 4.89 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1'), 4.52 (d, 1H, $J_{1',2''}$ 8.4 Hz, H-1''), 4.42 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.16 (dd, $J_{2',3'}$ 3.2 HZ, H-2'), 3.36 (s, 3H, OCH_3), 3.25 (s, 6H, $N(\underline{CH_3})_2C_2H_5$), 2.05 (s, 3H, NHAC), 1.39 (t, 3H, J_{vic} 7.0 Hz, $N(CH_3)_2CH_2\underline{CH_3}$), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 934.4 $(M+Na)^+$, 785.7 $(M-I+H)^+$, 785.7 $(M-I+H)^+$, 784.7 $(M-I)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-6-(2,4-dinitrophenyl amino)-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11i**).

To a solution of **11a** (0.95 mg, 1.42 μmol) in phosphate buffer (pH 7.2, 0.8 mL) was added 2,4-dinitrofluorobenzene (0.36 μL, 2.84 μmol). The reaction mixture was stirred at r.t. for 4 hrs and then taken to dryness. The residue was purified by preparative TLC which was developed using $CHCl_3$-MeOH-H_2O (65:35:8). The product was further purified as described for the preparation of **11a** to give **11i** (0.7 mg, 59%). ^1H NMR (D_2O, 400 MHz): δ 9.18 (dd, 1H, $J_{3,5}$ 1.5, $J_{3,6}$ 1.0 Hz, DNP C_3-H), 8.38 (dd, 1H, $J_{5,6}$ 9.8 Hz, DNP C_5-H), 7.26 (dd, 1H, DNP C_6-H), 4.95 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1'), 4.60 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.38 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.14 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.64 (s, 3H, OCH_3), 3.34 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.7 Hz, H-4'), 3.18

(dd, 1H, $J_{2,3}$ 8.0 Hz, H-2), 2.01 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 859.5 $(M+Na)^+$, 837.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-dansylamido-6-deoxy-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11j**).

To a solution of compound **11a** (1.49 mg, 2.22 μmol) in 0.01M $NaHCO_3$ (0.5 mL) was added a solution of dansyl chloride (0.9 mg, 3.33 μmol) in DMF (0.5 mL). The reaction mixture was stirred at r.t. for 4 hrs and then taken to dryness. The residue was purified by preparative TLC which was developed by $CHCl_3$-MeOH (7:3). The product was further purified as described for the preparation of **11a** to give **11j** (1.1 mg. 55%). ^1H NMR (D_2O, 400 MHz): δ 8.50 (dd, 1H, $J_{2,3}$ 8.0, $J_{2,4}$ 1.0 Hz, dansyl C_2-H), 8.49 (dd, 1H, $J_{3,4}$ 8.0 Hz, dansyl C_4-H), 8.40 (dd, 1H, $J_{7,8}$ 7.0, $J_{6,8}$ 1.0 Hz, dansyl C_8-H), 7.72 (dd, 1H, dansyl C_3-H), 7.71 (dd, 1H, $J_{6,7}$ 7.0 Hz, dansyl C_7-H), 7.40, (dd, 1H, dansyl C_6-H), 4.98 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.45 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.34 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.09 (dd, $J_{2',3'}$ 3.2 Hz, H-2'), 3.50 (s, 3H, OCH_3), 2.85 (s, 6H, $N(CH_3)_2$), 2.04 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 926.5 $(M+Na)^+$, 904.5 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-(2-diazo-3,3,3-trifluoropropanamido-6-deoxy-4-O-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**11k**).

A mixture of **11a** (5 mg, 7.45 μmol) and *p*-nitrophenyl 2-diazo-3,3,3-trifluoropropanoate (41 mg, 149 μmol) in dry DMF (1 mL) was stirred in dark at r.t. for 24 hrs and was concentrated. The residue was purified by chromatography on Iatrobeads, eluting first with CH_2Cl_2-MeOH (9:1) to remove excess *p*-nitrophenyl 2-diazo-3,3,3-trifluoropropanoate, then with $CHCl_3$-MeOH-H_2O (65:35:8). The product thus obtained was purified as described for the preparation of **11a** to provide the title compound **11k**

(5.5 mg, 91%). ^{1}H NMR (D_2O, 500 MHz): δ 4.842 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.555 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.428 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.085 (dd, 1H, $J_{2',3'}$ 3.6 Hz, H-2'), 3.878 (dd, 1H, $J_{3',4'}$ 9.6 Hz, H-3'), 3.718 (dd, 1H, $J_{2'',3''}$ 9.6 Hz, H-2''), 3.544 (s, 3H, OCH_3), 3.241 (dd, 1H, $J_{2,3}$ 8.0 Hz, H-2), 2.065 (s, 3H, Ac), 0.862 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (D_2O, 100.6 MHz): δ 176.54 ($NHCOCH_3$), 162.76 (DTP C=O), 103.13 (C-1), 100.60 (C-1''), 97.48 (C-1'), 79.81 (C-4'), 77.56 (C-2'), 76.88 (C-3), 76.67 (C-5''), 74.89 (C-5), 74.20 (C-3''), 73.91 (C-2), 71.64 (octyl C1), 70.96 (C-4), 70.69 (C-3'), 70.46 (C-4''), 66.61 (C-6), 61.42 (C-6''), 61.11 (OCH3), 56.25 (C-2''), 41.89 (C-6'), 31.93, 29.62, 29.29, 29.23, 25.90, 22.86 (octyl C2-C7), 23.13 (CH_3CO), 14.24 (octyl CH_3); FAB MS: m/z 845.5 $(M+K)^+$, 829.6 $(M+Na)^+$; Exact FAB MS for $C_{32}H_{53}N_4O_{16}F_3Na$, theoretical MS: 829.3306; Found: 829.3301 (std. deviation 1.4).

Methyl 4-azido-3-O-benzyl-4,6,-dideoxy-α-D-mannopyranoside (**41**).

A suspension of **40** (310 mg, 1.53 mmol) and dibutyltin oxide (456 mg, 1.83 mmol) in methanol (80 mL) was heated at reflux for 2 hrs. Solvent was removed under diminished pressure to get a colorless syrup. This dibutylstannylene derivative was dissolved in dry DMF (4 mL), benzyl bromide (272 μL, 2.29 mmol) was added and the solution was brought to 100 °C. The reaction mixture was stirred for 2 hrs until TLC showed complete disappearance of the starting material. The reaction mixture was then concentrated to dryness under diminished pressure. Chromatography (2:1 hexane-EtOAc) of the residue provided desired product **41** (294 mg, 80% based on consumed **40**) and recovered starting material **40** (55 mg, 18%); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45-7.30 (m, 5H, Ph), 4.708 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.707, 4.656 (2d, each 1H, J_{gem} 11.3 Hz, $PhCH_2$), 3.972 (ddd, 1H, H-2), 3.710 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.5 Hz, H-3), 3.710 (dq, 1H, H-5), 3.403 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-4), 3.342 (s, 3H, OCH_3), 2.410 (d, 1H, $J_{2,OH}$ 1.9 Hz, C2-OH), 1.329 (d, 3H, $J_{5,6}$ 6.1 Hz, 3H-6).

1,2-Di-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranose (**42**).

To a solution of **41** (290 mg, 0.989 mmol) in pyridine (3 mL) was added acetic anhydride (3 mL). The mixture was stirred at r.t. for 10 hrs and was taken to dryness *in vacuo*. The residue was dissolved in acetic anhydride-acetic acid-sulfuric acid (50:20:0.5, 7 mL) and was stirred at r.t. for 3 hrs, and then poured into ice-cold aqueous potassium carbonate. The product was extracted with dichloromethane. The extract was dried ($MgSO_4$) and concentrated to give **42** (358 mg, quant.) as a syrup; ^1H NMR ($CDCl_3$, 360 MHz): δ 7.40-7.25 (m, 5H, Ph), 6.02 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 5.33 (dd, 1H, $J_{2,3}$ 3.5 Hz, H-3), 4.71, 4.56 (2d, each 1H, J_{gem} 11.1 Hz, $PhCH_2$), 3.80 (dd, 1H, $J_{3,4}$ 10.0 Hz, H-3), 3.60 (dq, 1H, H-5), 3.47 (dd, 1H, $J_{4,5}$ 10.0 Hz, H-4), 2.13, 2.09 (2s, each 3H, 2 × Ac), 1.34 (d, 3H, $J_{5,6}$ 6.2 Hz, 3 H-6).

Octyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**43**).

To a mixture of **16** [88] (2.31 g, 4.10 mmol), AgOTf (1.32 g, 5.13 mmol) and molecular sieves 4 Å (4 g) in dry dichloromethane (10 mL) at -65 °C was added dropwise a solution of **34** [121] (1.14 g, 3.364 mmol) in CH_2Cl_2 (5 mL) under argon. The mixture was allowed to warm to r.t. within 2 hrs. After 1 h stirring, excess tetraethylammonium chloride (580 mg, 3.5 mmol) was added, and the mixture was stirred for another 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through a Celite bed. The filtrate was washed sequentially with 0.5% HCl, saturated $NaHCO_3$ and water, then dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography using hexane-EtOAc (2:1) as eluant to provide **43** (1.97 g, 67.6%) as a syrup; $[\alpha]_D$ +70.2° (*c* 0.6, $CHCl_3$); NMR data ($CDCl_3$): ^1H NMR (360 MHz), δ 7.38-7.20 (m, 20H, Ar-H), 5.394 (dd, 1H, $J_{2',3'}$ 3.2 Hz, $J_{1',2'}$ 1.9 Hz, H-2'), 4.980 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.970 (d, 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.880 (d, 1H, J_{gem} 11.1 Hz, $PhCH_2$), 4.778 (d, 1H, H-1'), 4.776 (d, 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.730 (d, 1H, J_{gem} 11.0

Hz, $PHCH_2$), 4.699 (d, 1H, J_{gem} 11.1 Hz, $PhCH_2$), 4.505 (d, 1H, J_{gem} 10.7 Hz, $PhCH_2$), 4.476 (d, 1H, J_{gem} 10.6 Hz, $PhCH_2$), 4.365 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.884 (dt, J_{gem} 9.4 Hz, J_{vic} 6.5 Hz, octyl C_1-H_a), 3.762 (dd, 1H, $J_{3',4'}$ 9.8 Hz, H-3'), 2.13 (s, 3H, Ac), 1.25 (d, 3H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 0.87 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz), δ 170.04 (CO), 138.51 (benzyl C_1), 138.47 (benzyl C_1), 137.98 (benzyl C_1), 137.18 (benzyl C_1), 103.54 (C-1, J_{CH} 156.9 Hz), 97.73 (C-1', J_{CH} 170.9 Hz), 75.68, 74.88, 74.77, 71.43 ($PhCH_2$), 70.11 (octyl C_1), 66.35 (C-6), 63.96 (C-4'), 31.81, 29.78, 29.43, 29.25, 26.18, 22.63 (octyl C_2-C_7), 20.94 ($CO\underline{CH_3}$), 18.46 (C-6'), 14.05 (octyl CH_3). Anal. Calc. for $C_{50}H_{63}O_{10}N_3$ (866.07): C, 69.34; H, 7.33; N, 4.85. Found: C, 69.62; H, 7.41; N, 4.73.

Octyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**44**).

Compound **43** (1.95 g, 2.25 mmol) was treated with methanolic NaOMe (0.05 N, 100 mL) at r.t. for 2 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left **44** as a syrup (1.81 g, 98%); ^1H NMR ($CDCl_3$, 360 MHz): δ 7.42-7.18 (m, 20H, Ar), 4.968 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.966 (d, 1H, J_{gem} 10.6 Hz, $PhCH_2$), 4.899 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.878 (d, 1H, $J_{1',2'}$ 1.2 Hz, H-1'), 4.788 (d, 1H, J_{gem} 10.6 Hz, $PhCH_2$), 4.731 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.682 (s, 2H, $PhCH_2$), 4.508 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.368 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.032 (ddd, 1H, $J_{2',3'}$ 4.5 Hz, H-2'), 3.891 (dt, 1H, J_{4gem} 9.5 Hz, J_{vic} 6.2 Hz, octyl C1-Ha), 3.775 (dd, 1H, $J_{3',4'}$ 11.0 Hz, H-3'), 2.340 (d, 1H, $J_{2',OH}$ 1.8 Hz, $C_{2'}$-OH), 1.24 (d, 3H, $J_{5',6'}$ 6.2 Hz, 3H-6'), 0.880 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

Octyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**45**).

A mixture of **44** (788 mg, 0.96 mmol), AgOTf (491 mg, 1.91 mmol), molecular sieve 4 Å (1.5 g), collidine (100 µL, 0.77 mmol) and dry CH_2Cl_2 (12 mL) was cooled to -78 °C. To the resulting mixture was added dropwise a solution of bromide **14** [120] (715 mg, 1.43 mmol) in dry CH_2Cl_2 (6 mL) under argon. The mixture was allowed to warm to r.t. over 1 h. After addition of excess tetraethylammonium chloride (250 mg, 1.5 mmol), the mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, saturated $NaHCO_3$ and water, then dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using hexane-EtOAc (2:1) as eluant to provide **45** (910 mg, 77%); $[\alpha]_D$ +31.8° (*c* 0.4, $CHCl_3$); NMR data ($CDCl_3$): ^1H (360 MHz), δ 7.825 (dd, 2H, J 5.4, 3.0 Hz, phthalimido C_3-H, C_6-H), 7.69 (dd, 2H, J 5.4, 3.0 Hz, phthalimido C_4-H, C_5-H), 7.41-7.13 (m, 20H, Ar), 5.869 (dd, 1H, $J_{2",3"}$ 10.9, $J_{3",4"}$ 9.2 Hz, H-3"), 5.432 (d, 1H, $J_{1",2"}$ 8.5 Hz, H-1"), 5.200 (dd, 1H, $J_{4",5"}$ 9.9 Hz, H-4"), 4.975 (d, 1H, J_{gem} 10.9 Hz, $PhCH_2$), 4.960 (d, 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.781 (d, 1H, J_{gem} 11.1 Hz, $PhCH_2$), 4.763 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.740 (d, 1H, J_{gem} 11.6 Hz, $PhCH_2$), 4.733 (d, 1H, J_{gem} 10.6 Hz, $PhCH_2$), 4.548 (d, 1H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.532 (d, 1H, J_{gem} 11.6 Hz, $PhCH_2$), 4.456 (dd, 1H, H-2"), 4.359 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.310 (d, 1H, J_{gem} 11.1 Hz, $PhCH_2$), 4.256 (dd, 1H, J_{gem} 12.3, $J_{5",6"}$ 2.4 Hz, H-6"), 4.127 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.058 (dt, 1H, J_{gem} 9.5 Hz, J_{vic} 6.3 Hz, octyl C1-H_a), 3.951 (ddd, 1H, $J_{4",5"}$ 10.2, $J_{5",6"a}$ 4.7, $J_{5",6"b}$ 2.5 Hz, H-5"), 3.200 (dd, 1H, $J_{4',5'}$ 9.9, $J_{3',4'}$ 9.8 Hz, H-4'), 3.099 (dq, 1H, $J_{5',6'}$ 6.0 Hz, $J_{4',5'}$ 9.8 Hz, H-5'), 2.078, 2.050, 1.894 (s, each 3H, 3 Ac), 0.875 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3), 0.545 (d, 3H, 3H-6'); ^{13}C (75.5 MHz): δ 170.70, 170.14, 169.39 (C=O), 138.49, 138.37, 138.03, 137.39 (benzyl C_1), 128.44, 128.39, 128.33, 128.04, 127.94, 127.71, 127.53 (Ar),

103.85 (C-1), 97.07 (C-1'), 96.07 (C-1"), 75.76, 74.74, 74.73, 70.68 (Ph$\underline{CH_2}$), 70.37 (octyl C_1), 66.57 (C-6), 63.35 (C-4'), 62.22 (C-6"), 54.33 (C-2"), 31.85, 29.83 29.49, 29.28, 26.20, 22.66 (octyl C_2-C_7), 20.76, 20.63, 20.51 (3 $\underline{CH_3}$CO), 17.91 (C-6'), 14.07 (octyl CH_3); Anal. Calcd for $C_{68}H_{80}N_4O_{18}$ (1241.41): C, 65.79; H, 6.50; N, 4.51. Found: C, 65.87; H, 6.64; N, 4.55.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**46**).

A solution of **45** (840 mg, 0.68 mmol) in butanol (120 mL) was added to ethylenediamine (24 mL). The mixture was stirred at 80 °C for 12 hrs. Concentration was followed by two successive additions and evaporations of toluene. The residue was not characterized but was dissolved in dry methanol (10 mL) to which acetic anhydride (10 mL) was added. After stirring at r.t. overnight, water (10 mL) was added, and the solution was concentrated. The residue was purified by column chromatography on Iatrobeads using CH_2Cl_2-MeOH (9:1) as eluant, to provide **46** (610 mg, 88%) as a colorless syrup; $[\alpha]_D$ +24.6° (*c* 1.3, $CHCl_3$); NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.40-7.10 (m, 20H, Ar-H), 4.853 (d, 1H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.529 (d, 1H, $J_{1",2"}$ 8.2 Hz, H-1"), 4.360 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.106 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 1.910 (s, 3H, Ac), 1.180 (d, 3H, $J_{5',6'}$ 6.5 Hz, 3 H-6'), 0.869 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C (75.5 MHz): δ 172.36 (C=O), 138.46, 138.38, 138.06, 136.97 (benzyl C_1), 103.83 (C-1), 99.09 (C-1"), 97.65 (C-1'), 70.67 (octyl C_1), 66.44 (C-6), 64.06 (C-4'), 62.40 (C-6"), 58.41 (C-2"), 31.86, 29.81, 29.47, 29.30, 26.20, 22.69 (octyl C_2-C_7), 23.58 ($\underline{CH_3}$CO), 18.73 (C-6'), 14.11 (octyl CH_3); Anal. Calcd for $C_{56}H_{74}O_{14}N_4$ (1027.23): C, 65.48; H, 7.26; N, 5.45. Found: C, 65.27; H, 7.22; N, 5.45.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4-amino-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12a**).

To a mixture of liq. NH_3 (80 mL) and *t*-butanol (0.3 mL) was added a solution of **46** (193 mg, 0.28 mmol) in THF (5 mL) at -78 °C. Small pieces of sodium were then added until the mixture remained blue and stirring was continued for 4 hrs. Ammonium chloride (solid) was added until the blue color disappeared. After evaporation of NH_3, the residue was dissolved in water. The aqueous solution was loaded onto a C-18 silica gel column (12 g). The column was eluated with water (3 × 30 mL) followed by methanol (100 mL). The methanolic eluate was taken to dryness and the residue was purified by chromatography on Iatrobeads using CH_2Cl_2-MeOH-H_2O (65:35:8) as eluant to provide the crude product. The crude product was absorbed onto a C-18 Sep-Pak cartridges (10 mg/cartridge) in water, the cartridges were washed with water (50 mL) and eluted with HPLC grade methanol (50 mL). Concentration of the eluate, dissolution of the residue in water, filtration through a Millex filter and lyophilization gave the target product **12a** (98 mg, 81%) as a white powder; $[\alpha]_D$ -22.3° (*c* 0.3, H_2O); NMR data (D_2O): ^1H (500 MHz): δ 4.938 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.617 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.479 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.178 (dd, $J_{2',3'}$ 3.0 Hz, H-2'), 4.023 (dd, 1H, $J_{3',4'}$ 10.0 Hz, H-3'), 3.990 (dd, 1H, J_{gem} 11.0, $J_{5,6a}$ 4.0 Hz, H-6a), 3.940 (dd, 1H, J_{gem} 12.0, $J_{5'',6''a}$ 2.2 Hz, H-6''a), 3.905 (dt, J_{gem} 12.0, J_{vic} 6.2 Hz, octyl C_1-H_a), 3.792 (dd, 1H, $J_{5'',6''b}$ 5.4 Hz, H-6''b), 3.780 (dd, 1H, $J_{5,6b}$ 6.0 Hz, H-6b), 3.752 (dd, 1H, $J_{2'',3''}$ 10.0 Hz, H-2''), 3.695 (dt, 1H, octyl C_1-H_b), 3.590 (m. 1H, H-5), 3.208 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.105 (dd, 1H, $J_{3',4'}$, 10.0 Hz, H-4'), 2.072 (s, 3H, $NHAc$), 1.643 (p, 2H, J_{vic} 7.0 Hz, octyl CH_2), 1.355 (d, 3H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C (100.6 MHz): δ 175.52 (C=O), 103.24 (C-1, J_{CH} 161.5 Hz), 100.24 (C-1'', J_{CH} 162.0 Hz), 97.96 (C-1', J_{CH} 171.6 Hz), 76.80 (C-3), 76.69 (C-5''), 75.77 (C-2'), 74.85 (C-5), 74.09 (C-3''), 73.92 (C-2), 71.62 (octyl C_1), 70.66 (C-4''), 70.15 (C-4), 67.72 (C-3'), 67.22 (C-5'), 66.71 (C-6), 61.33 (C-6''), 56.13 (C-2''), 55.12 (C-4'),

31.93, 29.58, 29.28, 29.20, 25.87, 22.84 (octyl C_2-C_7), 23.10 ($CO\underline{CH_3}$); 17.85 (C-6'), 14.26 (octyl CH_3). FAB MS: m/z 663.52 $(M+Na)^+$, 641.42 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4-acetamido-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12b**).

To a solution of **12a** (0.9 mg, 1.40 μmol) in dry methanol (0.5 mL) was added acetic anhydride (0.5 mL). The mixture was stirred at r.t. overnight, solvents were removed and the resulting residue was absorbed onto a Sep-Pak C-18 cartridge in water, the cartridge was washed with water (10 mL) followed by HPLC grade methanol (10 mL). Concentration of the eluate, followed by dissolution of the residue in water, filtration through a Millex filter and lyophilization afforded **12b** (0.96 mg, quant.); ^1H NMR (D_2O, 360 MHz): δ 4.920 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.635 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.485 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.155 (dd, 1H, $J_{2',3'}$ 3.1 Hz, H-2'), 3.280 (dd, 1H, $J_{2,3}$ 8.0 Hz, H-2), 2.095 (s, 3H, $NH\underline{Ac}$), 2.053 (s, 3H, $NH\underline{Ac}$), 1.200 (d, 3H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 705.87 $(M+Na)^+$, 683.85 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4-benzamido-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12c**).

To a solution of **12a** (2.0 mg, 3.12 μmol) in pyridine was added benzoyl chloride (28 μL, 220 μmol). The mixture was stirred at r.t. for 8 hrs, and then poured into ice water. The aqueous solution was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with 0.5 M HCl, saturated aq. $NaHCO_3$ and water, then dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography using hexane-EtOAc (1:1) as eluant to provide the perbenzoylated product (not characterized) which was treated with 0.05 N NaOMe/MeOH for 8 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of resin by filtration, and concentration left a residue which was

purified as described for the purification of **12b** to give **12c** (0.9 mg, 39%) as a white powder; ^{1}H NMR (D_2O, 360 MHz): δ 7.85 (dd, 2H, J 8.0, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.68 (ddd, 1H, J 8.0, 8.0, 1.5 Hz, benzoyl C_4-H), 7.58 (dd, 2H, J 8.0, 8.0 Hz, benzoyl C_3-H, C_5-H), 4.98 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.68 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.48 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.20 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 2.13 (s, 3H, NHAc), 1.27 (d, 3H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 767.66 $(M+Na)^+$, 745.48 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4,6-dideoxy-4-succinamido-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12d**).

A mixture of **12a** (2.5 mg, 3.9 μmol) and succinic anhydride (20 mg, 200 μmol) in dry methanol (3 mL) was refluxed for 4 hrs and then concentrated to dryness. The residue was purified by chromotography on Iatrobeads eluting first with CH_2Cl_2-MeOH (9:1) to remove excess succinic anhydride, then with CH_2Cl_2-MeOH (65:35:8) to obtain the crude product, which was purified as described for the preparation of **12b** to provide the title compound (1.7 mg, 59%) as a white powder; ^{1}H NMR (D_2O, 400 MHz): δ 4.88 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.61 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.46 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.12 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.91 (dd, 1H, $J_{3',4'}$ 10.5 Hz, H-3'), 3.26 (dd, 1H, $J_{2,3}$ 8.5 Hz, H-2), 2.61 and 2.56 (m, each 2H, $COCH_2CH_2COOH$), 2.08 (s, 3H, NHAc), 1.17 (d, 3H, $H5',6'$ 6.0 Hz, 3 H-6'), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 763.64 $(M+Na)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4,6-dideoxy-4-iodoacetamido-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12e**).

A mixture of **12a** (10 mg, 15.6 μmol) and iodoacetic anhydride (165 mg, 0.468 mmol) in dry methanol (1.5 mL) was stirred at r.t. for 24 hrs and concentrated. The residue was dissolved in water (1 mL) and stirred at r.t. for 30 min to destroy excess iodoacetic

anhydride. The product was then isolated on a C-18 Sep-Pak cartridge as described for the preparation of **12b**. Lyophilization after Millex filtration afforded **12e** (9 mg, 71%) as a white powder; ^{1}H NMR (D_2O, 400 MHz): δ 4.929 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.442 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.499 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.17 (dd, 1H, $J_{2',3'}$ 3.1 Hz, H-2'), 2.10 (s, 3H, NHAc), 1.66 (p, 2H, J 7.0 Hz, octyl CH_2), 1.24 (d, 3H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 175.51 (C=O), 173.19 (ICH_2CO), 103.15 (C-1), 100.06 (C-1''), 97.72 (C-1'), 71.60 (octyl C_1), 67.01 (C-6), 61.45 (C-6''), 56.23 (C-2''), 54.69 (C-4'), 31.94, 26.97, 29.31, 29.23, 25.98, 21.86 (octyl C_2-C_7), 23.12 (CH_3CO), 17.99 (C-6'), 14.26 (octyl CH_3), -1.48 (ICH_2); FAB MS: m/z 831.57 $(M+Na)^+$. 809.64 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4-acrylamido-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12f**).

To a solution of **12a** (2.3 mg, 3.59 μmol) in a mixture of aq. $NaHCO_3$ (0.1 N, 0.5 mL) and DMF (0.5 mL) was added acryloyl chloride (2.9 μL, 35.9 μmol). The resulting mixture was stirred at r.t. for 12 hrs and concentrated. The residue was purified by chromatography on Iatrobeads using CH_2Cl_2-MeOH-H_2O (65:35:4) as eluant to give the crude product. The crude product was further purified as described for the preparation of **12b** to give **12f** (1.3 mg, 52%) as a white powder; ^{1}H NMR (D_2O, 500 MHz): δ 6.274 (dd, 1H, J_{trans} 17.0 Hz, J_{cis} 9.5 Hz, $COCH=CH_2$), 6.218 (dd, 1H, J_{trans} 17.0 Hz, J_{gem} 2.5 Hz, acryloyl C_3-H_a), 5.800 (dd, 1H, J_{cis} 9.5, J_{gem} 2.5 Hz, acryloyl C_3-H_b), 4.906 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.618 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.456 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.138 (dd, 1H, $J_{2',3'}$ 3.1 Hz, H-2'), 3.962 (dd, 1H, $J_{3',4'}$ 10.5 Hz, H-3'), 3.262 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 2.072 (s, 3H, NHAc), 1.180 (d, 3H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3). FAB MS: m/z 717.24 $(M+Na)^+$, 695.35 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4,6-dideoxy-4-(1-thiophenylacetamido)-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12g**).

To a solution of **12e** (1.5 mg, 1.85 μmol) in 0.1 N $NaHCO_3$ (0.5 mL) was added thiophenol (0.77 μL, 11.3 μmol). The mixture was stirred at r.t. for 24 hrs and taken to dryness. The residue was purified as described for the preparation of **12b** to give **12g** (1.4 mg, 96%) as a white powder. ^1H NMR (D_2O, 400 MHz): δ 7.52-7.25 (m 5H, Ar-H), 4.91 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.62 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.48 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.13 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 2.08 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 813.76 $(M+Na)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4,6-dideoxy-4-(2,4-dinitrophenylamino)-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12h**).

To a solution of **12a** (2.3 mg, 3.59 μmol) in phosphate buffer (pH 7.2, 0.5 mL) was added 2,4-dinitrofluorobenzene (1.35 μL, 43.2 μmol). The mixture was stirred at r.t. for 24 hrs, then at 80 °C for additional 1 h until the starting material was completely consumed. After concentration, the residue was purified by chromatography on Iatrobeads eluting first with CH_2Cl_2-MeOH (9:1) to remove unreacted 2,4-dinitrofluorobenzene, then CH_2Cl_2-MeOH-H_2O (65:35:8) to yield crude product which was purified as described for the preparation of **12b** to give **12h** (2.8 mg, 97%) as a yellow powder; ^1H NMR (D_2O, 300 MHz): δ 9.14 (brs, 1H, Ph C_3-H), 8.35 (d, 1H, J 9.0 Hz, Ph C_5-H), 7.40 (d, 1H, J 9.0 Hz, Ph C_6-H), 5.19 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.67 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.45 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.21 (dd, 1H, $J_{2',3'}$ 3.1 Hz, H-2'), 2.31 (s, 3H, NHAc), 1.40 (d, 3H, $J_{5',6'}$, 6.0 Hz, 3 H-6'), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 829.71 $(M+Na)^+$, 807.74 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4-(2-diazo-3,3,3-trifluoropropanamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12i**).

To a mixture of **12a** (9.2 mg, 14.4 μmol), DMF (1 mL), and aq. $NaHCO_3$ (0.1N, 1 mL) was added 2-diazo-3,3,3-trifluoropropanoyl chloride (25 mg, 145 μmol). The reaction mixture was stirred in the dark at r.t. for 3 hrs, and then concentrated to dryness. The residue was subjected to chromatography on Iatrobeads using $CHCl_3$-MeOH-H_2O (65:35:5) as eluant to give the crude product. The crude product was further purified as described for the purification of **12b** to give the title product (8.0 mg, 72%); ^1H NMR (D_2O, 360 MHz): δ 4.998 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.698 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.505 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.268 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 2.100 (s, 3H, Ac), 1.070 (d, 3H, $J_{5',6'}$ 6.5 Hz, 3 H-6'), 0.865 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 799.0 $(M+Na)^+$, 777.1 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-4-(4-azidobenzamido)-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**12j**).

To a solution of **12a** (3.3 mg, 5.15 μmol) in aq. $NaHCO_3$ (0.1 N, 1 mL) was added *N*-hydroxysulfosuccimidyl 4-azidobenzoate (sulfo-HSAB, 15 mg, 52.2 μmol). The reaction mixture was stirred in the dark at r.t. for 48 hrs. The mixture was loaded onto a C-18 Sep-Pak cartridge, washed with water to remove the excess sulfo-HSAB, and then eluted with methanol to get the crude product. The crude product was purified by chromatography on Iatrobeads using $CHCl_3$-MeOH-H_2O (65:35:5) as eluant to give the TLC-homogeneous material. This material was further purified as described for the purification of compound **12b** to give **12j** as a white powder (3.0 mg, 74%); ^1H NMR (D_2O, 360 MHz): δ 7.825 (d, 2H, J 8.2 Hz, azidobenzoyl C_2-H, C_6-H), 7.125 (d, 2H, J 8.2 Hz, azidobenzoyl C_3-H, C_5-H), 4.916 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.630 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.435 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.145 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-

2'), 2.160 (s, 3H, Ac), 1.260 (d, 3H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.860 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz) δ 175.38 (C=O), 144.42 (Ar C_4), 130.57 (Ar C_1), 129.84 (Ar C_3/C_5), 119.78 (Ar C_2/C_6), 102.92 (C-1), 100.05 (C-1"), 97.50 (C-1'), 76.66 (C-3), 76.02 (C-5"), 75.28 (C-2'), 74.09 (C-5), 73.80 (C-3"), 71.25 (octyl C1), 70.71 (C-4"), 68.38 (C-4), 67.64 (C-3'), 61.41 (C-6"), 56.17 (C-2"), 54.95 (C-4'), 32.08, 29.90, 29.59, 29.38, 26.16, 22.91(octyl C2-C7), 23.17 ($\underline{CH_3}CO$), 18.12 (C-6'), 14.26 (octyl CH_3); FAB MS: m/z 808.3 $(M+Na)^+$, 786.3 $(M+H)^+$.

Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**48**).

A mixture of **44** (84 mg, 0.102 mmol), AgOTf (52.5 mg, 0.204 mmol), molecular sieves 4 Å (160 mg), collidine (11 μL, 82 μmol) and dry CH_2Cl_2 (3 mL) was cooled to -78 °C. To the resulting mixture was added dropwise a solution of bromide **47** [123] (85 mg, 0.153 mmol) in dry CH_2Cl_2 (2 mL) under argon. The mixture was allowed to warm to r.t. within 2 hrs and then stirred at r.t. for another 1 h. After the addition of excess tetraethylammonium chloride (33 mg, 0.20 mmol), the mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through celite. The filtrate was washed sequentially with 0.5% HCl, saturated $NaHCO_3$ and water, then dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography using hexane-EtOAc (4:1) as eluant to provide **48** (40.5 mg, 31%) as a syrup; $[\alpha]_D$ +41.0° (*c* 0.7, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 7.400-7.100 (m, 35H, Ar-H), 5.070 (dd, 1H, $J_{1",2"}$ 8.5, $J_{2",3"}$ 9.0 Hz, H-2"), 4.948 (d, 2H, J_{gem} 11.0 Hz, $PhCH_2$), 4.794 (d, 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.780 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.776 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.753 (d, 2H, J_{gem} 11.0 Hz, $PhCH_2$), 4.711 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.707 (d, 1H, $J_{1',2'}$ 2.0 Hz, H-1'), 4.661 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.537 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.520 (d, 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.504 (d, 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.460 (d, 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.360 (d, 1H, H-1"), 4.336

(d, 1H, Jgem 11.0 Hz, $PhCH_2$), 4.322 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.075 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.890 (dt, 1H, Jgem 9.5 hz, Jvic 6.5 Hz, octyl C_1-H_a), 3.632 (dd, 1H, $J_{3",4"}$ 9.5 Hz, H-3"), 3.480 (dt, 1H, octyl C_1-H_b), 3.418 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-4), 3.358 (dd, 1H, H-2), 1.939 (s, 3H, Ac), 1.138 (d, 3H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.845 (t, 3H, Jvic 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 169.25 (CO), 138.50, 138.40, 138.22, 138.04, 137.97, 137.83, 137.55 (benzyl C_1), 128.37, 128.33, 128.07, 127.92, 127.85, 127.76, 127.69, 127.55 (aromatic methine), 103.70 (C-1), 99.90 (C-1"), 97.67 (C-1'), 66.13 (C-6), 63.23 (C-4'), 31.80, 29.81, 29.44, 29.27, 26.19, 22.64 (octyl C_2-C_7), 18.44 (C-6'), 14.07 (octyl CH_3); Anal. Cacld for $C_{77}H_{79}N_3O_{15}$ (1298.60): C, 71.22; H, 7.06; N, 3.24. Found: C, 71.45; H, 6.89; N, 3.13.

Octyl 3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**49**).

Compound **48** (25.7 mg, 19.8 μmol) was treated with methanolic NaOMe (0.05 N, 2 mL) at r.t. for 10 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration and solvent evaporation left a residue that was purified by column chromatography using hexane-EtOAc (4:1) as eluant, yielding **49** as a syrup (18 mg, 70%). ^1H NMR (360 MHz, $CDCl_3$): δ 7.40-7.10 (m, 35H, Ar-H), 4.858 (d, 1H, $J_{1',2'}$ 2.0 Hz, H-1'), 4.483 (d, 2H, $J_{1",2"}$ 7.8 Hz, H-1"), 4.355 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.180 (dd, 1H, $H_{2',3'}$ 3.2 Hz, H-2'), 3.901 (dt, 1H, Jgem 9.5 Hz, Jvic 6.5 Hz, octyl C_1-H_a), 3.762 (dd, 1H, $J_{3',4'}$ 9.8 Hz, H-3'), 2.960 (d, 1H, $J_{2",OH}$ 1.8 Hz, $C_{2"}$-OH, D_2O exchangeable), 1.258 (d, 3H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 0.867 (t, 3H, Jvic 7.0 Hz, octyl CH_3).

Octyl β-D-glucopyranosyl-(1→2)-4-amino-4,6-dideoxy-α-D-mannopyranosyl]-(1→6)-β-D-glucopyranoside (**13**).

To a mixture of liq. NH_3 (15 mL) and *t*-butanol (0.1 mL) was added a solution of **46** (29 mg, 23.0 μmol) in THF (1 mL) at -78 °C. Small pieces of sodium were then added until the mixture remained blue and stirring was continued for 6 hrs. Ammonium chloride (solid) was added until the blue color disappeared. After evaporation of NH_3, the residue was purified as described for the preparation of **12a**. Lyophilization gave **13** (6.5 mg, 47%) as a white powder; $[\alpha]_D$ +15.2° (*c* 0.6, CH_3OH), ^1H NMR (D_2O, 500 MHz): δ 4.981 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1'), 4.506 (d, 1H, $J_{1'',2''}$ 7.5 Hz, H-1''), 4.453 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.096 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.935 (dd, 1H, J_{gem} 11.1 Hz, $J_{5,6a}$ 4.5 Hz, H-6a), 3.905 (dd, 1H, J_{gem} 11.5 Hz, $J_{5'',6''b}$ 2.5 Hz, H-6''b), 3.877 (dt, 1H, J_{gem} 10.5 Hz, J_{vic} 7.0 Hz, octyl C_1-H_a), 3.790 (dd, 1H, $J_{5,6b}$ 2.5 Hz, H-6b), 3.775 (dq, $J_{4',5'}$ 10.0 Hz, $J_{5',6'}$ 6.2 Hz, H-5'), 3.730 (dd, 1H, $J_{5'',6''a}$ 5.0 Hz, H-6''a), 3.680 (ddd, 1H, J_{gem} 10.5 Hz, J_{vic} 7.0 Hz, octyl C_1-H_b), 3.580 (ddd, $J_{4,5}$ 9.5 Hz, H-5), 3.495 (dd, 1H, $J_{3'',4''}$, $J_{2'',3''}$ 9.0 Hz, H-3''), 3.465 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.434 (dd, 1H, H-4), 3.412 (dd, 1H, $J_{4'',5''}$ 9.5 Hz, H-4''), 3.342 (dd, 1H, H-2''), 3.232 (dd, 1H, H-2), 2.848 (dd, 1H, H-4'), 1.622 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 1.302 (d, 1H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 0.860 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3). ^{13}C NMR: δ 103.18 (C-1, $J_{C\text{-}1,H\text{-}1}$ 161.2 Hz), 102.62 (C-1'', $J_{C\text{-}1'',H\text{-}1''}$ 161.3 Hz), 98.79 (C-1', $J_{C\text{-}1',H\text{-}1'}$ 171.8 Hz), 77.52 (C-2'), 76.84 (C-3), 76.75 (C-5''), 76.30 (C-3''), 74.77 (C-5), 73.91 (C-2), 73.57 (C-2''), 71.60 (octyl C_1), 70.26 (C-4), 70.23 (C-4''), 69.74 (C-3'), 69.42 (C-5'), 66.57 (C-6), 61.40 (C-6''), 55.10 (C-4'), 31.90 (octyl C_2), 29.58, 29.22, 29.17, 25.85, 22.82 (octyl C_3-C_7), 17.64 (C-6'), 14.23 (octyl CH_3); FAB MS (m/z): 622.3 $(M+Na)^+$, 600.3 $(M+H)^+$.

Octyl 3,4,6-tri-O-benzyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**56**).

A mixture of **16** [88] (600 mg, 1.07 mmol) and trifluoromethanesulfonic acid (17.6 μL, 0.2 mmol) in dry THF (20 mL) was cooled to -78 °C. To the resulting mixture was added dropwise a solution of 1,2-anhydro-3,4,6-tri-*O*-benzyl-α-D-glucopyranose **54** [128] (432 mg, 1.0 mmol) in dry THF (10 mL). The reaction mixture was stirred at -78 °C for 2 hrs, then warmed to r.t. and stirred for 10 hrs. The reaction mixture was then concentrated and the residue was dissolved in CH_2Cl_2 (20 mL). The CH_2Cl_2 solution was washed with saturated $NaHCO_3$ and water, then dried over anhydrous $MgSO_4$, filtered and concentrated. The purification of the residue by column chromatography (hexane-EtOAc 2:1) provided **56** (170 mg, 24% based on consumed alcohol), **57** (330 mg, 47%) and recovered alcohol **16** (200 mg, 33%); $[\alpha]_D$ +57.3° (*c* 0.7, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 7.40-7.10 (m, 30H, Ar-H), 4.968, 4.960, 4.952, (3d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.945 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.884 (d, 1H J_{gem} 11.0 Hz, $PhCH_2$), 4.838 (d, 2H, J_{gem} 10.8 Hz, $PhCH_2$), 4.782, 4.717 (2d, each 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.605, 4.572 (2d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.483 (d, 1H, J_{gem} 10.1 Hz, $PhCH_2$), 4.450 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.406 (d, 1H $J_{1,2}$ 7.8 Hz, H-1), 3.822 (ddd, 1H, $J_{2',3'}$ 10.0 Hz, $J_{2',OH}$ 2.0 Hz, H-2'), 3.422 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 0.865 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 138.50, 138.43, 137.98 (benzyl C_1), 128.44, 128.36, 128.25, 128.10, 128.00, 127.88, 127.76, 127.74, 127.64, 127.59, 127.53 (Ar), 103.54 (C-1), 99.19 (C-1'), 75.69, 75.23, 74.94, 74.86, 74.83, 73.42 ($PhCH_2$), 70.21 (octyl C_1), 68.41 (C-6"), 67.03 (C-6), 31.82, 29.77, 29.42, 29.25, 26.19, 22.64 (octyl C_2-C_7), 14.07 (octyl CH_3); Anal. Calcd for $C_{62}H_{74}O_{11}$ (995.27): C, 74.82; H, 7.49; Found: C, 74.90; H, 7.61.

Octyl 3,4,6-tri-O-benzyl-2-O-trifluoromethanesulfonyl-α-D-glucopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**58**).

To a mixture of compound **56** (115 mg, 0.116 mmol) in dry pyridine (2 mL) at -22° C was added trifluoromethanesulfonic anhydride (85 μL, 0.505 mmol). The reaction mixture was allowed to warm to r.t. and stirred then for 1 h. After addition of ice cold 1 M HCl (10 mL), the reaction mixture was stirred for 30 minutes and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated $NaHCO_3$ and water, dried over anhydrous $MgSO_4$. Filtration followed by evaporation of the CH_2Cl_2 gave the title product (130 mg, 100%) as a syrup. ^1H NMR (360 MHz, $CDCl_3$): δ 7.40-7.05 (m, 30H, Ar-H), 5.175 (d, 1H, $J_{1',2'}$ 3.5 Hz, H-1'), 4.955, 4.950, 4.936, 4.886, 4.845, 4.780, 4.775, 4.755 (8d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.735 (dd, 1H, $J_{2',3'}$ 9.5 Hz, H-2'), 4.705 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.595 (d, 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.575 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.445 (d, 1H, J_{gem} 12.0 Hz, $PhCH_2$), 4.388 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.050 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3'), 0.865 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

Octyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-2-deoxy-2-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**59**).

To a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose **53** [125] (105 mg, 0.288 mmol) in dry THF (2 mL) was added sodium hydride (60% suspension in oil, 13 mg, 0.318 mmol). The mixture was stirred, resulting in the evolution of bubbles. After a white solid (sodium salt) was produced, the THF was evaporated using a stream of argon. To the resulting residue was added a solution of **58** (130 mg, 0.115 mmol) in dry DMF (2 mL). The reaction mixture was stirred at r.t. for 12 hrs, and methanol (2 mL) was added to quench the reaction. The reaction mixture was concentrated to dryness and the residue was dissolved in CH_2Cl_2 (10 mL). The CH_2Cl_2 solution was washed with water, dried over $MgSO_4$, filtered and concentrated. Purification of the residue by column chromatography using hexane-EtOAc (2:1) as eluant gave the title compound (38 mg, 25%); ^1H NMR (360

MHz, $CDCl_3$): δ 5.245 (dd, 1H, $J_{2",3"}$, $J_{3",4"}$ 9.2 Hz, H-3"), 5.136 (dd, 1H, $J_{1',2'}$ 2.0 Hz, H-1'), 5.104 (dd, 1H, $J_{4",5"}$ 9.5 Hz, H-4"), 5.065 (dd, 1H, $J_{1",2"}$ 9.6 Hz, H-2"), 4.978, 4.946, 4.855, 4.819, 4.805, 4.799, 4.776, 4.710 (8d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.475 (d, 1H, H-1"), 4.400 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.368 (d, 1H, J_{gem} 10.5 Hz, $PhCH_2$), 4.225 (dd, 1H, J_{gem} 12.5 Hz, $J_{5",6"a}$ 5.5 Hz, H-6"a), 4.142 (dd, 1H, $J_{5",6"b}$ 2.0 Hz, H-6"b), 3.382 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 2.006, 1.985, 1.975, 1.965 (4s, each 3H, 4 Ac), 0.870 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 174.22, 170.58, 170.07, 169.36 (C=O), 138.53, 138.42, 138.22, 138.06, 137.89 (benzyl C_1), 128.53, 128.38, 128.32, 128.20, 128.15, 128.03, 127.94, 127.89, 127.75, 127.63, 127.52 (Ar), 103.75 (C-1), 101.27 (C-1"), 87.60 (C-1'), 70.48 (octyl C_1), 68.81 (C-6"), 66.09 (C-6), 62.33 (C-6'), 47.03 (C-2'), 31.66, 29.72, 29.46, 29.30, 26.16, 22.68 (octyl C_2-C_7), 20.72 ($\underline{CH}_3CO$), 14.10 (octyl CH_3); ESI MS: m/z 1363.7 $(M+Na)^+$; Exact ESI MS for $C_{76}H_{92}O_{19}SNa$, theoretical MS: 1363.58512; Found: 1363.58515.

Octyl β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-2-deoxy-2-thio-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**60**).

Compound **59** (30 mg, 22.4 μmol) was treated with methanolic NaOMe (0.05 N, 3 mL) at r.t. for 12 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left a residue which was purified by column chromatography (5% MeOH in CH_2Cl_2) and preparative TLC (5% MeOH in CH_2Cl_2) to give compound **60** as a syrup (20 mg, 76%); $[\alpha]_D$ +30.9° (*c* 1.3, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 5.101 (d, 1H, $J_{1',2'}$ 2.0 Hz, H-1'), 4.965, 4.960, 4.875, 4.805, 4.792, 4.785, 4.735, 4.695 (8d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.425 (d, 1H, J_{gem} 12.5 Hz, $PhCH_2$), 4.388 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.290 (d, 1H, $J_{1",2"}$ 9.5 Hz, H-1"), 4.162 (dd, 1H, $J_{2',3'}$ 4.0 Hz, $J_{3',4'}$ 8.5 Hz, H-3'), 3.943 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.2 Hz, octyl C_1-Ha), 3.452 (dd, 1H, H-2'), 2.575 (d, 1H, J 1.0 Hz, OH), 2.520 (s,

1H, OH), 2.515 (d, 1H J 1.2 Hz, OH), 2.315 (dd, 1H, J 6.0, 5.5 Hz, $C_{6"}$-OH), 0.870 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 138.41, 138.22, 138.04, 137.00 (benzyl C_1), 128.74, 128.59, 128.41, 128.37, 128.31, 128.12 127.94, 127.70, 127.66, 127.52 (Ar), 103.73 (C-1), 101.92 (C-1"), 85.09 (C-1'), 75.78, 74.89, 74.82, 74.55, 73.71, 73.34 (Ph$\underline{CH_2}$), 71.99 (octyl C_1), 68.51 (C-6'), 65.66 (C-6), 62.39 (C-6"), 45.88 (C-2'), 31.81, 29.68, 29.42, 29.24, 26.12, 22.64 (octyl C_2-C_7), 14.07 (octyl CH_3): FAB MS: m/z 1211.3 $(M+K)^+$, 1196.4 $(M+Na)^+$.

Octyl β-D-glucopyranosyl-(1→2)-2-deoxy-2-thio-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**50**).

A solution of **60** (13 mg, 11.1 μmol) in THF (0.5 mL) was added to a mixture of liq. NH_3 (10 mL, distilled over Na) and *t*-butanol (50 μL) at -78 °C. Small pieces of sodium were added until the reaction mixture remained blue, and the mixture was stirred at -78 °C for 4 hrs. Ammonium chloride (solid) was then added until the blue color disappeared. The liquid NH_3 was allowed to evaporate slowly and the remaining solution was concentrated to dryness. The residue was dissolved with Milli-Q water. The aqueous solution was loaded on a C-18 Sep-Pak cartridge. The cartridge was washed with water (2 × 10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness. The residue was dissolved with Milli-Q water, passed through a Millex filter and the filtrate was lyophilized to give **50** (5.5 mg, 79%); $[\alpha]_D$ +1.1° (*c* 0.7, CH_3OH); ^1H NMR (500 MHz, D_2O): δ 5.147 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1'), 4.546 (d, 1H, $J_{1,2}$ 10.0 Hz, H-1"), 4.449 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.175 (dd, 1H, $J_{2',3'}$ 4.5 Hz, $J_{3',4'}$ 9.5 Hz, H-3'), 3.905 (dd, 1H, J_{gem} 11.2 Hz, $J_{5,6a}$ 5.4 Hz, H-6a), 3.883 (dd, 1H, J_{gem} 12.5 Hz, $J_{5',6'a}$ 2.2 Hz, H-6'a), 3.843 (dd, 1H, J_{gem} 11.9 Hz, $J_{5",6"a}$ 2.5 Hz, H-6"a), 3.780 (dd, 1H, $J_{5,6b}$ 2.0 Hz, H-6b), 3.720 (dd, 1H, $J_{5',6'b}$ 6.9 Hz, H-6'b), 3.670 (dt, 1H J_{gem} 10.5 Hz, J_{vic} 7.0 Hz, octyl C_1-H_b), 3.630 (dd, 1H, H-2'), 3.580 (m, 1H, H-5), 3.505 (dd, 1H, $J_{4',5'}$ 9.6 Hz, H-4'), 3.478 (dd, 1H, $J_{2",3"}$, $J_{3",4"}$ 9.6 Hz, H-3"),

3.445 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.422 (dd, 1H, $J_{4",5"}$ 9.5 Hz, H-4"), 3.360 (dd, 1H, H-2"), 3.254 (dd, 1H, H-2), 1.618 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 1.38-1.24 (m, 10H, octyl CH_2), 0.860 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (125.7 MHz, D_2O): δ 103.17 (C-1, $J_{C-1,H-1}$ 160.4 Hz), 101.26 (C-1', $J_{C-1',H-1'}$ 174.3 Hz), 85.99 (C-1", $J_{C-1",H-1"}$ 156.4 Hz), 80.81 (C-3), 77.84 (C-5"), 76.89 (C-3"), 74.91 (C-5), 73.97 (C-5'), 73.81 (C-2), 73.06 (C-2"), 71.62 (octyl C_1), 70.36 (C-4), 70.20 (C-4"), 69.73 (C-3'), 68.61 (C-4'), 66.67 (C-6), 61.61 (C-6'), 61.45 (C-6"), 51.12 (C-2'), 31.93, 29.63, 29.27, 29.21, 25.89, 22.86 (octyl C_2-C_7), 14.26 (octyl CH_3): FAB MS: m/z 671.0 $(M+K)^+$, 655.0 $(M+Na)^+$, 633.0 $(M+H)^+$.

Octyl 2,3,4-tri-O-benzyl-6-O-methanesulfonyl-β-D-glucopyranoside (**66**).

To a solution of **16** [88] (213 mg, 0.370 mmol) in pyridine at -25 °C was added dropwise methanesulfonyl chloride (292 μL, 3.790 mmol). The reaction mixture was warmed to r.t. over 30 min and stirred at r.t. for 4 hrs. The mixture was then poured into ice cold 0.5 N aqueous HCl and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed by saturated $NaHCO_3$ and water. Concentration gave **66** (234 mg, 97%). ^1H NMR (360 MHz, $CDCl_3$): δ 7.350-7.100 (m, 15H, Ar-H), 4.960, 4.943, 4.890, 4.798, 4.710, 4.618 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.455 (dd, 1H, J_{gem} 11.5 Hz, $J_{5,6a}$ 1.0 Hz, H-6a), 4.413 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.328 (dd, 1H, $J_{5,6b}$ 4.0 Hz, H-6b), 3.898 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.5 Hz, octyl C_1-H_a), 3.668 (ddd, 1H, $J_{4,5}$ 9.5 Hz, H-5), 3.588 (dt, 1H, octyl C_1-H_b), 3.418 (dd, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.032 (s, 3H, Ms), 1.633 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 1.410-1.190 (m, 10 H, octyl CH_2), 0.878 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

Octyl 6-S-acetyl-2,3,4-tri-O-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (**67**).

A mixture of **66** (230 mg, 0.359 mmol) and potassium thioacetate (433 mg, 3.79 mmol) in dry DMF (15 mL) was stirred at r.t. for 16 hrs. TLC showed at that point that the

reaction was not yet complete. The reaction mixture was then stirred at 40 °C for an additional 2 hrs and concentrated to dryness. The residue was dissolved in CH_2Cl_2, washed with water and concentrated. The residue was purified by silica gel chromatography using hexane-EtOAc (6:1) as eluant to give **67** (195 mg, 88%). ^1H NMR (360 MHz, $CDCl_3$): δ 7.350-7.100 (m, 15H, Ar-H), 4.945, 4.928, 4.878, 4.780, 4.705, 4.635 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.358 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 3.905 (dt, 1H, J_{gem} 9.8 Hz, J_{vic} 7.0 Hz, octyl C_1-H_a), 3.631 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.596 (dd, 1H, J_{gem} 14.0 Hz, $J_{5,6a}$ 2.7 Hz, H-6a), 3.572 (dt, 1H, octyl C_1-H_b), 3.460-3.310 (m, 3H, H-2, H-4, H-5), 2.970 (dd, 1H, $J_{5,6b}$ 7.5 Hz, H-6b), 2.360 (s, 3H, Ac), 1.648 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 0.888 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 194.92 (C=O), 138.50, 138.43, 137.83 (benzyl C_1), 128.47, 128.37, 128.34, 128.26, 128.10, 127.88, 127.64 (Ar), 103.48 (C-1), 75.69, 75.14, 74.76 ($PhCH_2$), 31.82 (C-6), 31.06 (octyl C_2), 30.47 (CH_3CO), 29.76, 29.38, 29.24, 26.15, 22.66 (octyl C_3-C_7), 14.08 (octyl CH_3); Anal. Calcd for $C_{37}H_{48}O_6S$ (620.84): C, 71.58; H, 7.79; S, 5.16. Found: C, 71.27; H, 8.15; S, 5.52.

Octyl 2,3,4-tri-O-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (**62**).

Compound **67** (133 mg, 0.214 mmol) was treated with methanolic NaOMe (0.05 N, 45 mL) at r.t. for 30 min. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration of the filtrate gave **62** (124 mg, 96%); ^1H NMR (360 MHz, $CDCl_3$): δ 7.40-7.10 (m, 15H, Ar-H), 4.955, 4.948, 4.890, 4.785, 4.715, 4.617 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.420 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 3.948 (dt, 1H, J_{gem} 9.6 Hz, J_{vic} 6.5 Hz, octyl C_1-H_a), 3.655 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.562 (dt, 1H, octyl C_1-H_b), 3.471 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-4), 3.428 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.366 (ddd, 1H, $J_{5,6a}$ 2.8 Hz, $J_{5,6b}$ 7.5 Hz, H-5), 2.886 (ddd, 1H, J_{gem} 14.0 Hz, $J_{6a,SH}$ 9.5 Hz, H-6a), 2.631 (ddd, 1H, $J_{6b,SH}$ 7.6 Hz, H-6b), 1.695 (dd, 1H, SH,

D_2O exchangeable), 1.662 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 0.881 (t, 3H, J_{vic} 7.0 Hz, CH_3).

2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-glucose (**70**).

To a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide (**47**) [123] (313 mg, 0.563 mmol) in acetone (4 mL) at 0 °C was added water (1 drop) and silver carbonate (192 mg, 0.698 mmol). The reaction mixture was vigorously stirred, warmed to r.t. and stirred overnight. The reaction mixture was filtered through a Celite bed which was washed with acetone. The acetone filtrate was concentrated and, the residue was purified by chromatography using hexane-EtOAc (2:1) as eluant to afford **70**. Compound **70** was not characterized but was used directly for the preparation of imidate **71**.

O-(2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-trichloroacetimidate (**71**).

To a solution of **70** (64 mg, 0.130 mmol) in dry CH_2Cl_2 was added NaH (60% suspension in oil, 5.2 mg, 0.130 mmol) and trichloroacetonitrile (60 μL). The reaction mixture was stirred at r.t. for 2 hrs and was concentrated. The residue was purified by chromatography using hexane-ether-Et_3N (50:50:1) as eluent to afford imidate **71** (50 mg, 60%); ^1H NMR (360 MHz, $CDCl_3$): δ 8.580 (s, 1H, NH), 7.300-7.100 (m, 15H, Ar-H), 6.527 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.071 (dd, 1H, $J_{2,3}$ 9.6 Hz, H-2), 4.863 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.838 (d, 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.767 (d, 1H, J_{gem} 11.5 Hz, $PhCH_2$), 4.635 (d, 1H, J_{gem} 11.8 Hz, $PhCH_2$), 4.578 (d, 1H, J_{gem} 10.8 Hz, $PhCH_2$), 4.504 (d, 1H, J_{gem} 11.8 Hz, $PhCH_2$), 4.097 (dd, 1H, $J_{3,4}$ 9.5 Hz, H-3), 4.015 (ddd, 1H, $J_{4,5}$ 9.5 Hz, H-5), 3.882 (dd, 1H, H-4), 3.815 (dd, 1H, J_{gem} 11.2 Hz, $J_{5,6a}$ 3.5 Hz, H-6a), 3.697 (dd, 1H, $J_{5,6b}$ 2.0 Hz, H-6b), 1.920 (s, 3H, Ac).

Octyl 3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (**72**).

To a solution of **62** (124 mg, 0.214 mmol) in dry THF (5 mL) was added sodium hydride (60% suspension in oil, 13 mg, 0.321 mmol). The resulting mixture was stirred at r.t. for 30 min. After a white precipitate (sodium salt) was produced, the THF was removed using a stream of argon. The residuum sodium salt was dissolved with DMF (2ml) and was added dropwise to a solution of 1,2-anhydro-3,4,6-tri-O-benzyl-β-D-mannose **61** [131] (85 mg, 0.195 mmol) in DMF (1 mL). The reaction mixture was stirred at r.t. for 1h. Methanol (1 mL) was added to the reaction mixture and the reaction mixture was then concentrated and the residue was purified by column chromatography using hexane-EtOAc (3:1) as eluent to give **72** (94 mg, 63%); $[\alpha]_D$ +83.4° (*c* 0.5, CH_2Cl_2); ^1H NMR (360 MHz, $CDCl_3$): δ 5.442 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.955, 4.940, 4.850, 4.815, 4.763, 4.707 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.615 (d, 1H, J_{gem} 12.2 Hz, $PhCH_2$), 4.565, 4.507 (2d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.438 (d, 1H, J_{gem} 12.2 Hz, $PhCH_2$), 4.378 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.137 (ddd, 1H, $J_{2',3'}$ 3.2 Hz, $J_{2',OH}$ 2.7 Hz, H-2'), 4.118 (ddd, 1H, H-5'), 3.934 (dt, J_{gem} 10.0 Hz, Jvic 6.5 Hz, octyl C_1-H_a), 3.912 (dd, 1H, $J_{4',5'}$, $J_{3',4'}$ 9.5 Hz, H-4'), 3.845 (dd, 1H, H-3'), 3.753 (dd, 1H, J_{gem} 11.8 Hz, $J_{5',6'a}$ 4.5 Hz, H-6'a), 3.628 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.614 (dd, 1H, $J_{5',6'b}$ 2.0 Hz, H-6'b), 3.500 (dt, 1H, octyl C_1-H_b), 3.485 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-4), 3.480 (m, 1H, H-5), 3.413 (dd, 1H, H-2), 3.067 (dd, J_{gem} 14.0 Hz, $J_{5,6a}$ 2.5 Hz, H-6a), 2.775 (dd, 1H, $J_{5,6b}$ 6.0 Hz, H-6b), 2.569 (d, 1H, $C_{2'}$-OH), 0.869 (t, 3H, Jvic 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 138.58, 138.49, 138.35, 138.23, 137.72 (benzyl C_1), 128.55, 128.45, 128.33, 128.10, 128.00, 127.90, 127.86, 127.63, 127.54 (aromatic methine), 103.51 (C-1), 84.53 (C-1'), 75.63, 75.15, 75.07, 74.76, 73.40, 72.04 ($PhCH_2$), 70.15 (octyl C_1), 68.88 (C-6'), 31.89 (C-6), 31.83, 29.77, 29.43, 29.25, 26.19, 22.66 (octyl C_2-C_7), 14.09 (octyl CH_3); Anal. Calcd for $C_{62}H_{74}O_{10}S$ (1011.33): C, 73.63; H, 7.38, S, 3.17. Found: C, 73.18; H, 7.44; S, 3.30.

Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (**73**).

A mixture of **71** (48 mg, 75.4 μmol), **72** (66 mg, 65.2 μmol), powdered molecular sieves 4Å (60 mg) and CH_2Cl_2 (3 mL) was stirred at r.t. for 30 min. To the mixture was added a solution of TMSOTf (1.45 μL, 7.5 μmol) in CH_2Cl_2 (150 μL) under argon. After stirring for 1 h, Et_3N (0.1 mL) was added and the mixture was filtered through Celite and concentrated. The residue was purified by chromatography using hexane-EtOAc (3:1) as eluent to afford **73** (44 mg, 88% based on consumed alcohol) and recovered alcohol **72** (32 mg, 48%); $[\alpha]_D$ +37.7° (*c* 1.2, CH_2Cl_2): ^1H NMR (360 MHz, $CDCl_3$): δ 5.400 (d, 1H, $J_{1',2'}$ 1.2 Hz, H-1'), 5.146 (dd, 1H, $J_{1'',2''}$ 8.5 Hz, $J_{2'',3''}$ 9.0 Hz, H-2''), 4.468 (d, 1H, H-1''), 4.392 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.269 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.078 (ddd, 1H, $J_{4',5'}$ 9.6 Hz, $J_{5',6'a}$ 6.4 Hz, $J_{5',6'b}$ 2.0 Hz, H-5'), 3.967 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.5 Hz, octyl C_1-H_a), 3.822 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3'), 3.624 (dd, 1H, $J_{3,4}$ 9.5 Hz, H-3), 3.527 (dt, 1H, octyl C_1-H_b), 3.439 (dd, $J_{2,3}$ 9.2 Hz, H-2), 3.102 (dd, 1H, J_{gem} 14.2 Hz, $J_{5,6a}$ 2.2 Hz, H-6a), 2.819 (dd, 1H, $J_{5,6b}$ 5.4 Hz, H-6b), 1.917 (s, 3H, Ac), 0.857 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 169.38 (C=O), 138.58, 138.56, 138.45, 138.33, 138.27, 138.14, 137.96, 137.93 (benzyl C_1), 128.43, 128.37, 128.34, 128.28, 128.23, 128.05, 127.99, 127.97, 127.84, 127.81, 127.69, 127.66, 127.59, 127.44 (aromatic methine), 103.69 (C-1), 99.05 (C-1''), 84.55 (C-1'), 75.61, 75.27, 75.10, 75.00, 74.83, 74.81, 73.59, 72.92, 70.48 (Ph$\underline{CH_2}$), 70.26 (octyl C_1), 69.71 (C-6''), 69.48 (C-6'), 31.82 (C-6), 31.81, 29.84, 29.49, 29.27, 26.23, 22.63 (octyl C2-C7), 20.90 ($\underline{CH_3}CO$), 14.06 (octyl CH_3); Anal. Calcd for $C_{91}H_{104}O_{16}S$ (1485.99): C, 73.56; H, 7.05; S, 2.16. Found: C, 73.59, H, 7.14, S, 2.10.

Octyl 3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-6-deoxy-6-thio-β-D-glucopyranoside (**74**).

Compound **73** (24 mg, 16.2 μmol) was treated with methanolic NaOMe (0.05 N, 25 mL) at r.t. for 8 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left a residue that was purified by column chromatography (hexane-EtOAc 2:1) to give **74** as a syrup (21 mg, 92%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.538 (d, 1H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.438 (d, 1H, $J_{1'',2''}$ 7.8 Hz, H-1''), 4.372 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.332 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.115 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4'), 4.032 (ddd, 1H, $J_{5',6'a}$ 4.5 Hz, $J_{5',6'b}$ 2.0 Hz, H-5'), 3.982 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.5 Hz, octyl C_1-H_a), 3.882 (dd, 1H, H-3'), 3.425 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2), 3.380 (br-s, 1H, $C_{2''}$-OH), 3.065 (dd, 1H, J_{gem} 14.5 Hz, $J_{5,6a}$ 2.0 Hz, H-6a), 2.829 (dd, 1H, $J_{5,6b}$ 5.0 Hz, H-6b), 0.845 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 139.00, 138.55, 138.52, 138.47, 138.33, 138.18, 138.04, 137.97, 137.73 (benzyl C_1), 128.46, 128.34, 128.28, 128.25, 128.01, 127.93, 127.87, 127.81, 127.76, 127.64, 127.58, 127.54, 127.42 (aromatic methine), 103.67 (C-1), 100.65 (C-1''), 84.49 (C-1'), 75.59, 75.14, 75.08, 74.98, 74.86, 74.79, 73.54, 73.29, 71.67 ($PhCH_2$), 70.42 (octyl C_1), 69.54 (C-6''), 68.69 (C-6'), 32.42 (C-6), 31.81, 29.81, 19.45, 29.25, 26.21, 22.63 (octyl C_2-C_7), 14.07 (octyl CH_3).

Octyl β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-6-deoxy-6-thio-β-D-glucopyranoside **(51).**

A solution of 74 (18 mg, 12.5 μmol) in THF (1.0 mL) was added to a mixture of liq. NH_3 (10 mL, distilled over Na) and *t*-butanol (50 μL) at -78 °C. Small pieces of sodium were added until the reaction mixture remained blue, and the mixture was stirred at -78 °C for 4 hrs. Ammonium chloride (solid) was then added until the blue color disappeared. The liquid NH_3 was allowed to evaporate slowly and the remaining solution was concentrated to dryness. The residue was then purified as described for the preparation

of **50**. Lyophilization from water gave **51** as a white powder (6.5 mg, 82%); $[\alpha]_D$ +54.6° (*c* 0.5, CH_3OH); ^1H NMR (500 MHz, D_2O): δ 5.503 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1'), 4.501 (d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1''), 4.459 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.240 (dd, 1H, $J_{2',3'}$ 3.5 Hz, H-2'), 4.005 (ddd, 1H, $J_{4',5'}$ 9.5 Hz, $J_{5',6'a}$ 3.2 Hz, $J_{5',6'b}$ 6.0 Hz, H-5'), 3.895 (dd, 1H, J_{gem} 12.0 Hz, $J_{5'',6''a}$ 2.0 Hz, H-6''a), 3.890 (dd, 1H, J_{gem} 12.0 Hz, $J_{5',6'a}$ 6.5 Hz, H-6'a), 3.865 (dd, 1H, $J_{5',6'b}$ 2.5 Hz, H-6'b), 3.860 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3'), 3.845 (dt, 1H, J_{gem} 10.5 Hz, J_{vic} 6.5 Hz, octyl C_1-H_a), 3.745 (dd, 1H, $J_{5'',6''b}$ 5.4 Hz, H-6''b), 3.738 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$ 9.5 Hz, H-4'), 3.708 (dt, 1H, octyl C1-H_b), 3.610 (ddd, 1H, $J_{4,5}$ 9.5 Hz, $J_{5,6a}$ 2.7 Hz, $J_{5,6b}$ 8.0 Hz, H-5), 3.490 (ddd, 1H, $J_{4'',5''}$ 9.5 Hz, H-5''), 3.472 (dd, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.430 (dd, 1H, H-4''), 3.385 (dd, 1H, H-4), 3.355 (dd, 1H, $J_{2'',3''}$ 9.5 Hz, H-2''), 3.265 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.202 (dd, J_{gem} 14.4 Hz, $J_{5,6a}$ 2.7 Hz, H-6a), 2.804 (dd, 1H, $J_{5,6b}$ 8.0 Hz, H-6b), 1.638 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 1.400-1.250 (m, 10H, 5 octyl CH_2), 0.870 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 103.21 (C-1, $J_{C\text{-}1,H\text{-}1}$ 160.6 Hz), 101.82 (C-1'', $J_{C\text{-}1'',H\text{-}1''}$ 161.1 Hz), 82.72 (C-1', $J_{C\text{-}1',H\text{-}1'}$ 167.9 Hz, C-1'), 79.36 (C-2'), 76.78 (C-3), 76.49 (C-5''), 76.34 (C-3''), 74.85 (C-5), 74.06 (C-2), 73.86 (C-5'), 73.47 (C-2''), 73.14 (C-4), 71.84 (octyl C_1), 71.06 (C-3'), 70.29 (C-4''), 67.86 (C-4'), 61.46 (C-6''), 61.16 (C-6'), 32.36 (C-6), 31.94, 29.66, 29.25, 29.17, 25.85, 22.84 (octyl C_2-C_7), 14.24 (octyl CH_3); FAB MS: m/z 671.1 $(M+K)^+$, 655.2 $(M+Na)^+$, 633.3 $(M+H)^+$; Exact FAB MS for $C_{26}H_{48}O_{15}SNa$, theoretical MS: 655.2612; Found: 655.2620 (std. deviation 1.3).

Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**76**).

A mixture of **16** (490 mg, 0.871 mmol), silver trifluoromethanesulfonate (336 mg, 1.31 mmol), molecular sieves 4 Å (1.0 g), collidine (92 μL, 0.697 mmol) and dry CH_2Cl_2 (7 mL) was cooled to -78 °C. To the resulting mixture was added dropwise a solution of 2-

O-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl bromide **75** [123] (543 mg, 0.978 mmol) in dry CH_2Cl_2 (1 mL) under argon. The mixture was allowed to warm to r.t. within 1 h and then stirred at r.t. for another 1h. After addition of excess tetraethylammonium chloride (165 mg, 1.0 mmol), the mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, saturated $NaHCO_3$ and water, and then dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using toluene-EtOAc (10:1) as eluant to provide compound **76** (763 mg, 85%) as a syrup; $[\alpha]_D$ +20.4° (*c* 2.3, CH_2Cl_2); ^1H NMR (360 MHz, $CDCl_3$): δ 7.40-7.10 (m, 30H, Ar-H), 5.428 (dd, 1H, $J_{1',2'}$ 1.9 Hz, $J_{2',3'}$ 2.9 Hz, H-2'), 4.962, 4.955, 4.855, 4.854, 4.775, 4.723, 4.692, 4.655, 4.520, 4.500, 4.450, 4.430 (12d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.902 (d, 1H, H-1'), 4.355 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.942 (dd, 1H, $J_{3',4'}$ 9.2 Hz, H-3'), 3.873 (dd, 1H, $J_{4',5'}$ 9.6 Hz, H-4'), 3.788 (ddd, 1H, $J_{5',6'a}$ 4.0 Hz, $J_{5',6'b}$ 1.6 Hz, H-5'), 3.670 (dd, 1H, J_{gem} 11.0 Hz, $J_{5',6'a}$ 4.0 Hz, H-6'a), 3.593 (dd, 1H, $J_{5',6'b}$ 1.6 Hz, H-6'b), 2.142 (s, 3H, Ac), 0.872 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3). ^{13}C NMR (100.6 MHz): δ 170.24 (C=O), 138.54, 138.48, 138.43, 138.16, 137.96, 137.79 (aromatic quart.), 128.29, 128.17, 128.09, 128.03, 127.79, 127.67, 127.64, 127,57, 127,44, 127.32 (aromatic CH), 103.45 (C-1), 97.73 (C-1'), 75.58, 74.89, 74.79, 74.68, 73.18, 71.51 (6× benzylic), 69,97 (octyl C_1), 66.04 (C-6), 31.75, 29.64, 29.35, 29.16, 26.13, 22.58 (octyl C_2-C_7), 21.03 (Ac), 14.02 (octyl CH_3); Anal. Calcd for $C_{64}H_{74}O_{12}$ (1037.31): C, 74.11; H, 7.39. Found: C, 73.80; H, 7.03.

Octyl 3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**77**).

Compound **76** (753 mg, 0.725 mmol) was treated with methanolic NaOMe (0.05 N, 30 mL) at r.t. for 10 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left a residue that was purified by column

chromatography (toluene-EtOAc 10:1) to give **77** as a white solid (660 mg, 92%); ^{1}H NMR ($CDCl_3$, 360 MHz): δ 7.40-7.10 (m, 30H, Ar-H), 4.988 (d, 1H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.680 (d, 1H, $J_{1'',2''}$ 7.8 Hz, H-1''), 4.358 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.082 (ddd, 1H, $J_{2',3'}$ 4.5 Hz, H-2'), 3.895 (dt, 1H, J_{gem} 9.5 Hz, J_{vic} 6.6 Hz, octyl C_1-H_a), 3.855 (dd, 1H, J_{gem} 12.1 Hz, $J_{5,6a}$ 4.9 Hz, H-6a), 3.465 (dt, 1H, octyl C_1-H_b), 2.410 (d, 1H, $J_{2',OH}$ 3.0 Hz, OH-2'), 0.873 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**78**).

A mixture of **77** (651 mg, 0.654 mmol), silver trifluoromethanesulfonate (420 mg, 1.64 mmol), molecular sieves 4 Å (1.2 g), collidine (69 μL, 0.523 mmol) and dry CH_2Cl_2 (8 mL) was cooled to -78 °C. To the resulting mixture was added dropwise a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide **47** [123] (727 mg, 1.308 mmol) in dry CH_2Cl_2 (1 mL) under argon. The mixture was allowed to warm to r.t. within 1 h. After addition of excess tetraethylammonium chloride (232 mg, 1.4 mmol), the mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through celite. The filtrate was washed sequentially with 0.5% HCl, saturated $NaHCO_3$ and water, and then dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using hexane-EtOAc (4:1) as eluant to provide compound **78** (523 mg, 55%) as a syrup; $[\alpha]_D$ +6.7° (*c* 0.6, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40-7.10 (m, 45H, Ar-H), 5.105 (dd, 1H, $J_{1'',2''}$ 8.3 Hz, $J_{2'',3''}$ 8.5 Hz, H-2''), 4.855 (d, 1H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.438 (d, 1H, H-1''), 4.368 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.173 (dd, 1H, $J_{2',3'}$ 3.5 Hz, H-2'), 3.812 (dd, 1H, $J_{3',4'}$ 9.0 Hz, H-3'), 1.935 (s, 3H, Ac), 0.850 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 169.28 (C=O), 103.64 (J_{CH} 158.1 Hz, C-1), 99.66 (J_{CH} 158.2 Hz, C-1''), 97.84 (J_{CH} 169.0 Hz, C-1'), 70.21 (octyl C1), 65.83 (C-6), 20.80 (Ac), 14.00 (octyl CH_3).

Octyl 3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (79).

Compound **78** (492 mg, 0.335 mmol) was treated with methanolic NaOMe (0.05 N, 80 mL) at r.t. for 10 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left a residue that was purified by column chromatography (hexane-EtOAc 2:1) to give **79** as a syrup (450 mg, 95%); ^1H NMR ($CDCl_3$, 360 MHz): δ 7.40-7.10 (m, 45H, Ar-H), 4.935 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.436 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.345 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.245 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.488 (d, 1H, $J_{2'',OH}$ 3.2 Hz, OH), 0.865 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

Octyl 2-O-allyl-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**80**).

To a mixture of **79** (450 mg, 0.315 mmol), sodium hydride (60% suspension in oil, 19 mg, 0.473 mmol) and dry DMF (12 mL) was added dropwise allyl bromide (35 μL, 0.410 mmol). The reaction mixture was stirred at r.t. for 8 hrs. Methanol was added to the reaction mixture to destroy excess NaH, after which it was concentrated to dryness under diminished pressure. Chromatography (4:1 hexane-EtOAc) of the residue afforded **80** (373 mg, 81%) as a syrup; $[\alpha]_D$ +5.5° (*c* 0.6, CH_2Cl_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 7.400-7.050 (m, 45H, Ar-H), 5.930 (dddd, 1H, $J_{1a,2} = J_{1b,2} = 6.5$ Hz, allyl C_2-H), 5.270 (dddd, 1H, $J_{2,3a}$ 18.0 Hz, $J_{3a,3b} = J_{1a,3a} = J_{1b,3a} = 1.0$ Hz, allyl C_3-H_a), 5.040 (dddd, 1H, $J_{2,3b}$ 10.8 Hz, $J_{3a,3b} = J_{1a,3b} = J_{1b,3b} = 1.0$ Hz, allyl C_3-H_b), 4.965 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.442 (d, 1H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.428 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.285 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 0.887 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (85.5 MHz): δ 135.39 (allyl C2), 117.06 (allyl C3), 103.68 (C-1), 102.83 (C-1''), 98.34 (C-1'), 70.27 (octyl C1), 66.10 (C-6), 14.06 (octyl CH_3); Anal. Calcd for $C_{92}H_{106}O_{16}$ (1467.86): C, 75.28; H, 7.28. Found: C, 75.21; H, 7.37.

Octyl 2-O-(2-hydroxylethyl)-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**81**).

A solution of **80** (285 mg, 0.194 mmol) in CH_2Cl_2-MeOH (10:1, 30 mL) at -78 °C was passed through O_3 for 6 min, and then to it was added dimethyl sulfide (1.5 mL). The reaction mixture was stirred for 30 min, after which sodium borohydride (74 mg, 1.94 mmol) was added. After stirring at r.t. for 3 more hrs, water (0.3 mL) was added to the reaction mixture to destroy excess $NaBH_4$. The reaction mixture was then concentrated to dryness. The residue was dissolved in CH_2Cl_2, washed with water, dried ($MgSO_4$) and concentrated. The residue was purified by chromatography using hexane-EtOAc (3:1) as eluant to afford **81** (186 mg, 65%) as a syrup; $[\alpha]_D$ +4.4° (*c* 0.6, CH_2Cl_2); ^1H NMR ($CDCl_3$, 500 MHz): δ 7.40-7.05 (m, 45H, Ar-H), 4.987 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.415 (d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1''), 4.328 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.282 (d, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.885 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.8 Hz, octyl C1-H_a), 3.840 (dd, 1H, $J_{3',4'}$ 9.8 Hz, H-3'), 3.430 (dt, 1H, octyl C1-H_b), 3.135 (dd, 1H, J 9.6, 3.7 Hz, OH), 0.853 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 103.71 (C-1), 102.18 (C-1''), 98.01 (C-1'), 70.37 (octyl C1), 68.35 ($O\underline{CH_2}CH_2OH$), 66.40 (C-6), 62.20 ($OCH_2\underline{CH_2}OH$), 14.08 (octyl CH_3); Anal. Calcd for $C_{91}H_{106}O_{17}$ (1471.8): C, 74.26; H, 7.26. Found: C, 74.62; H, 6.95.

Octyl 2-O-(2-acetathioethyl)-3,4,6-tri-O-benzyl-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**83**).

To a solution of **81** (22 mg, 15 μmol) in pyridine (1 mL) at -15 °C was added dropwise methanesulfonyl chloride (24 μL, 302 μmol). The reaction mixture was warmed to r.t. and stirred at r.t. for 2 hrs. The reaction mixture was poured into ice cold 0.5 N aq. HCl, then the HCl aq. solution was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. The residue was not characterized, but was dissolved in DMF (1 mL), and potassium

thioacetate (17 mg, 150 μmol) was added. The reaction mixture was then stirred at r.t. for 48 hrs. TLC showed that the reaction was not yet complete. The reaction mixture was stirred at 60 °C for an additional 2 hrs and taken to dryness. The residue was dissolved in CH_2Cl_2, washed with water, dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography using hexane-EtOAc (3:1) as eluant to afford **83** (20 mg, 86%) as a syrup; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40-7.05 (m, 45H, Ar-H), 4.968 (d, 1H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.476 (d, 1H, $J_{1'',2''}$ 7.5 Hz, H-1''), 4.360 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.255 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.028 (t, 2H, J_{vic} 6.2 Hz, -$OCH_2\underline{CH_2}SAc$), 2.180 (s, 3H, Ac), 0.870 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

Octyl 2-O-(2-thioethyl)-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**52**).

A solution of **83** (17 mg, 11.1 μmol) in THF (0.5 mL) was added to a mixture of liq. NH_3 (10 mL, distilled over Na) and *t*-butanol (50 μL) at -78 °C. Small pieces of sodium were added until the reaction mixture remained blue, and the mixture was stirred at -78 °C for 24 hrs. Ammonium chloride was then added until the blue color disappeared. The liquid NH_3 was allowed to evaporate slowly and the remaining solution was concentrated to dryness. The residue was dissolved with Milli-Q water. The aqueous solution was loaded onto a C-18 Sep-Pak cartridge. The cartridge was washed with water (2 × 10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness. The residue was dissolved with Milli-Q water, passed through a Millex filter and the filtrate was lyophilized to give **52** (5.0 mg, 67%) as white powder; ^{1}H NMR (D_2O, 500 MHz): δ 5.049 (d, 1H, $J_{1'2'}$ 1.0 Hz, H-1'), 4.558 (d, 1H, $J_{1'',2''}$ 7.6 Hz, H-1''), 4.449 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.180 (dt, 1H, J_{gem} 11.0 Hz, J_{vic} 6.6 Hz, -$OC\underline{H_a}H_bCH_2SH$), 4.170 (dd, 1H, $J_{2',3'}$ 3.0 Hz, H-2'), 3.998 (dt, 1H, J_{gem} 11.0 Hz, J_{vic} 6.6 Hz, -$OCH_a\underline{H_b}CH_2SH$), 3.996 (dt, 1H, J_{gem} 10.9 Hz, J_{vic} 6.6 Hz, octyl C1-H_a), 3.750 (dd, 1H, $J_{3',4'}$ 9.0 Hz, H-3'), 3.580 (m, 1H, H-5), 3.518 (dd, 1H, $J_{3'',4''}$ 9.0 Hz, H-3''), 3.472

(dd, 1H, $J_{3,4}$ 9.0 Hz, H-3), 3.263 (dd, 1H, $J_{2,3}$ 8.5 Hz, H-2), 3.218 (dd, 1H, $J_{2'',3''}$ 8.8 Hz, H-2"), 3.012 (t, 2H, J_{vic} 6.6 Hz, -$CH_2\underline{CH_2}$SH), 1.640 (p, 2H, J_{vic} 7.0 Hz, octyl CH_2), 1.400-1.240 (m, 10H, octyl CH_2), 0.878 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 715.6 $(M+K)^+$, 699.4 $(M+Na)^+$, 677.5 $(M+H)^+$; Exact FAB MS for $C_{28}H_{52}SNa$, theoretical MS: 699.2874; Found: 699.2879 (std. deviation 3.2).

Preparative enzymatic synthesis of octyl β-D-glucopyranosyl-(1→2)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-2-deoxy-2-thio-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**84**).

A mixture of **50** (0.49 mg, 0.774 μmol), UDP-GlcNAc (2 mg), cloned rat kidney GlcNAcT-V [84] (0.5 mL, 1.48 mU/mL) and buffer (170 mM sodium cacodylate buffer, pH 6.5, 70% glycerol, 35 mM EDTA, and 3.5 mg/mL BSA, in a total volume of 200 μL) was incubated at r.t. for 48 hrs. The mixture was diluted with Milli-Q water and loaded onto a C-18 Sep-Pak cartridge. The cartridge was eluted with water (3 × 10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness, then the residue was dissolved in Milli-Q water and passed through a Millex filter. Compound **84** was obtained as a white powder in 100% yield after lyophilization of the filtrate. ^{1}H NMR (500 MHz, D_2O): δ 5.125 (d, 1H, $J_{1',2'}$ 0.5 Hz, H-1'), 4.526 (d, 1H, $J_{1'',2''}$ 10.5 Hz, H-1"), 4.454 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.524 (d, 1H, $J_{1''',2'''}$ 8.0 Hz, H-1'"), 4.165 (dd, 1H, $J_{2',3'}$ 4.5 Hz, $J_{3',4'}$ 9.8 Hz, H-3'), 3.612 (dd, 1H, H-2'), 3.379 (dd, 1H, $J_{2'',3''}$ 9.5 Hz, H-2"), 3.258 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 2.090 (s, 3H, Ac), 1.625 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 1.400-1.240 (m, 10H, octyl CH_2), 0.86 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 858.1 $(M+Na)^+$.

Preparative enzymatic synthesis of octyl β-D-glucopyranosyl-(1→2)-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→6)-6-deoxy-6-thio-β-D-glucopyranoside (**85**).

A mixture of **51** (0.50 mg, 0.789 μmol), UDP-GlcNAc (1.5 mg), partially purified GlcNAcT-V (1.5 mL, 1 mU/mL) from hamster kidneys [108] and buffer (150 mM sodium cacodylate buffer, pH 6.5, 60% glycerol, 30 mM EDTA, and 3 mg/mL BSA, in a total volume of 500 μL) was incubated at r.t. for 72 hrs. The mixture was diluted with Milli-Q water and loaded onto a C-18 Sep-Pak cartridge. The cartridge was eluted with water (3 × 10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness, then the residue was purified by chromatography on Iatrobeads using $CHCl_3$-MeOH-H_2O (65:35:8) as eluant to give the crude product. The crude product was dissolved in Milli-Q water, and was loaded onto a C-18 Sep-Pak cartridge. The cartridge was washed with water (10 mL) followed by methanol (10 mL). The methanolic eluate was concentrated to dryness, then the residue was dissolved in Milli-Q water, and passed through a Millex filter. Compound **85** was obtained as a white powder in 100% yield after lyophilization of the filtrate. ^{1}H NMR (500 MHz, D_2O): δ 5.481 (d, 1H, $J_{1',2'}$ 1.0 Hz, H-1'), 4.522 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.476 (d, 1H, $J_{1''',2'''}$ 7.6 Hz, H-1'''), 4.453 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.220 (dd, 1H, $J_{2',3'}$ 3.5 Hz, H-2'), 4.152 (dd, 1H, J_{gem} 11.0 Hz, $J_{5',6'a}$ 2.4 Hz, H-6'a), 4.105 (ddd, 1H, $J_{4',5'}$ 9.5 Hz, $J_{5',6'b}$ 4.8 Hz, H-5'), 3.935 (d, 1H, J_{gem} 12.0 Hz, $J_{5'',6''a}$ 2.0 Hz, H-6''a), 3.828 (dd, 1H, $J_{3',4'}$ 9.5 Hz, H-3'), 3.605 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6a}$ 3.0 Hz, $J_{5,6b}$ 8.0 Hz, H-5), 3.260 (dd, 1H, $J_{2,3}$ 9.5 Hz, H-2), 3.172 (dd, 1H, J_{gem} 14.3 Hz, $J_{5,6a}$ 3.0 Hz, H-6a), 2.800 (dd, 1H, $J_{5,6b}$ 8.0 Hz, H-6b), 2.070 (s, 3H, Ac), 1.635 (p, 2H, J_{vic} 7.0 Hz, octyl C_2-H), 0.870 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); FAB MS: m/z 874.2 $(M+K)^+$, 858.2 $(M+Na)^+$, 836.3 $(M+H)^+$.

(6S) - [6-^{2}H] 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**91**).

A solution of (6S)-[6-^{2}H]-1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**90**) [145] (320 mg, 0.818 mmol) in 30% HBr/HOAc (5 mL) was stirred at r.t. for 2 hrs, and then concentrated to dryness. The residue was dissolved in CH_2Cl_2 and washed with successively with cold water, saturated aq. $NaHCO_3$, and again with cooled water and then dried over $MgSO_4$. Removal of the solvent gave a syrup which was recrystallize from ether to give **91** as a colorless crystal (323 mg, 96%). ^{1}H NMR ($CDCl_3$, 360 MHz): δ 6.615 (d, 1H, $J_{1,2}$ 4.2 Hz, H-1), 5.563 (dd, 1H, $J_{2,3} = J_{3,4}$ 9.6 Hz, H-3), 5.162 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-4), 4.840 (dd, 1H, H-2), 4.315 (d, 1H, $J_{5,6R}$ 4.5 Hz, H-6*pro*R), 4.298 (dd, 1H, H-5), 2.106, 2.100, 2.008, 2.038 (4s, each 3H, 4 × Ac).

(6S) - [6-^{2}H] Octyl β-D-glucopyranoside (**93**).

To a mixture of 1-octanol (513 μL, 3.26 mmol), Ag_2O (248 mg, 1.07 mmol), molecular sieves 4 Å (500 mg) and anhydrous ether (8 mL) was added dropwise a solution of **91** (323 mg, 0.784 mmol) in ether (2 mL). The reaction mixture was stirred at r.t. for 2 hrs, filtered through a Celite bed, and washed with ether. The filtrate was concentrated to give a syrup. The syrup was not characterized, but was treated with methanolic NaOMe (0.05 N, 10 mL) at r.t. for 10 hrs. The reaction mixture was neutralized with acetic acid, and then concentrated to dryness under diminished pressure. The residue was subjected to column chromatography using CH_2Cl_2-MeOH (9:1) as eluant to provide the product. The product was further purified by recrystallization in ether to afford **93** (188 mg, 82% in two steps) as a colorless crystal. ^{1}H NMR (360 MHz, D_2O): δ 4.445 (d, 12H, $J_{1,2}$ 8.0 Hz, H-1), 3.910 (dt, 1H, J_{gem} 9.9 Hz, J_{vic} 6.8 Hz, octyl C1-H_a), 3.693 (d, 1H, $J_{5,6R}$ 6.0 Hz, H-6*pro*R), 3.668 (dt, 1H, octyl C1-H_b), 3.478 (dd, 1H, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 8.9 Hz, H-3), 3.436 (ddd, 1H, $J_{4,5}$ 9.7 Hz, H-5), 3.425 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-4), 3.246 (dd, 1H, H-2), 1.528 (p, 2H, J_{vic} 7.0 Hz, octyl CH_2), 1.250-1.400 (m, 12H, 6 × octyl CH_2), 0.860 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz) δ 101.99 (C-1), 75.66 (C-3, C-

5), 72.98 (C-2), 70.54 (octyl C1), 60.30 (C-6), 30.90, 28.54, 28.26, 28.18, 24.86, 21.82 (octyl C2-C7), 13.20 (octyl CH_3).

(6S) - [6-^{2}H] Octyl 2,3,4-Tri-O-benzyl-β-D-glucopyranoside (**96S**).

A mixture of **93** (182 mg, 0.620 mmol), chloro-4-methoxyphenyl diphenyl methane (287 mg, 0.930 mmol) and pyridine (3 mL) was stirred at r.t. for 10 hrs, and then concentrated, the residue was purified by column chromatography using CH_2Cl_2-MeOH (19:1) as eluant, and the eluate was taken to dryness. The residue was not characterized, but was directly benzylated with sodium hydride (142 mg, 3.543 mmol, 60% dispersion in oil) and benzyl bromide (379 µL, 3.186 mmol) in DMF (5 mL) at r.t. for 12 hrs. Solvent evaporation and purification of the major product by chromatography using hexane-EtOAc (10:1) as eluant gave a chromatographically homogeneous material. This material was then treated with 80% aqueous acetic acid at r.t. for 24 hrs. Solvent evaporation followed by chromatographic purification of the residue using hexane-EtOAc (4:1) as eluant to afford **96S** as a white solid (222 mg, 64% yield in three steps); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.928 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.921 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.845 (d, 1H, J_{gem} 11.0Hz, $PhCH_2$), 4.792 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.706 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.620 (d, 1H, Jgem 10.8 Hz, $PhCH_2$), 4.420 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.904 (dt, J_{gem} 9.6 Hz, J_{vic} 6.5 Hz, octyl C_1-H_a), 3.661 (d, 1H, $J_{5,6R}$ 4.9 Hz, H-6*pro*R), 3.651 (dd, 1H, $J_{3,4}$ 9.2 Hz, $J_{2,3}$ 9.0 Hz, H-3), 3.538 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-4), 3.528 (dt, 1H, J_{gem} 9.6 Hz, J_{vic} 6.5 Hz, octyl C_1-H_b), 3.396 (dd, 1H, $J_{2,3}$ 9.2 Hz, H-2), 3.342 (dd, 1H, H-5), 1.635 (m, 2H, octyl C_2-H), 1.378 (p, 2H, J_{vic} 7.0 Hz, octyl CH2), 1.325-1.200 (m, 8H, octyl CH2), 0.865 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR ($CDCl_3$, 75.5 Hz): δ 138.58, 138.44, 138.02 (benzyl C_1), 128.49, 128.38, 128.10, 127.89, 127.69, 127.63 (aromatic methine), 103.74 (C-1), 84.54 (C-3), 82.34 (C-2), 77.67 (C-4), 75.68 ($PhCH_2$), 75.08 ($PhCH_2$), 74.95 (C-5), 74.88 ($PhCH_2$), 70.46 (octyl C_1), 61.69 (C-6), 31.83, 29.81, 29.41, 29.24, 26.15, 22.67 (octyl C2-C7), 14.10 (octyl

CH_3); Anal. Calcd for $C_{35}H_{45}DO_6$ (563,76): C, 74.57; H, 8.40. Found: C, 74.72; H, 8.38.

(6R) - [6-^{2}H] Octyl 6-O-benzoyl-2,3,4-Tri-O-benzyl-β-D-glucopyranoside (**97**).

To a mixture of **96S** (87 mg, 0.154 mmol), triphenylphosphine (202 mg, 0.772 mmol), benzoic acid (95 mg, 0.772 mmol) and THF (6 mL) was added dropwise diethyl azodicarboxylate (172 µL, 0.926 mmol). The reaction mixture was stirred at r.t. for 30 min and was taken to dryness. The residue was dissolved in CH_2Cl_2 and was washed with 1 M HCl, saturated $NaHCO_3$ and water. The CH_2Cl_2 solution was then dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography using hexane-EtOAc (4:1) as eluant to give **97** (95 mg, 92%) as a white solid; ^{1}H NMR ($CDCl_3$, 360 MHz): δ 8.040 (dd, 2H, J 8.1, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.568 (dddd, 1H, J 7.2, 7.2, 1.5, 1.5 Hz, benzoyl C_4-H), 7.439 (ddd, 2H, J 8.1, 7.2 Hz, benzoyl C_3-H, C_5-H), 4.978 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.974 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.890 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.818 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.745 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.602 (d, 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.598 (d, 1H, $J_{5,6S}$ 2.0 Hz, H-6*pro*S), 4.440 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 3.916 (dt, 1H, J_{gem} 9.6, J_{vic} 6.8 Hz, octyl C_1-H_a), 3.725 (dd, 1H, $J_{3,4}$ 9.2 Hz, H-3), 3.662 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-4), 3.622 (dd, 1H, H-5), 3.533 (dt, 1H, octyl C_1-H_b), 1.680 (m, 2H, octyl CH_2), 0.872 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 166.25 (C=O), 138.43, 137.70, 130.00 (aromatic quart.), 133.00, 129.70, 128.47, 128.42, 128.37, 128.33, 128.10, 127.98, 127.94, 127.70 (aromatic CH), 103.75 (C-1), 84.74 (C-3), 82.29 (C-2), 77.76 (C-4), 75.84, 75.13, 74.82 (3 × benzylic), 72.92 (C-5), 70.29 (octyl C1), 63.34 (C-6), 31.79, 29.72, 29.35, 29.22, 26.11, 22.62 (octyl C2-C6), 14.06 (octyl CH_3).

(6R) - [6-^{2}H] Octyl 2,3,4-Tri-O-benzyl-β-D-glucopyranoside (**96R**).

Compound **97** (92 mg, 0.138 mmol) was treated with methanolic NaOMe (0.05 N, 10 mL) at r.t. for 10 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration of the filtrate left a residue that was purified by column chromatography (hexane-EtOAc 2:1) to give **96R** as a white solid (74 mg, 95%); ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.40-7.20 (m, 15H, Ar-H), 4.943, 4.936, 4.861, 4.806, 4.718, 4.634 (6d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.434 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.929 (dt, 1H, J_{gem} 9.5 Hz, J_{vic} 6.6 Hz, octyl C_1-H_a), 3.854 (dd, 1H, $J_{5,6S}$ 2.7 Hz, $J_{6S,\ OH}$ 5.8 Hz, H-6*pro*S), 3.675 (dd, 1H, $J_{3,4}$ 9.2 Hz, $J_{2,3}$ 9.0 Hz, H-3), 3.568 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-4), 3.549 (dt, 1H, J_{gem} 9.6 Hz, J_{vic} 6.9 Hz, octyl C_1-H_b), 3.419 (dd, 1H, H-2), 3.363 (dd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6S}$ 2.7 Hz, H-5), 1.884 (d, 1H, C_6-OH), 1.648 (m, 2H, octyl CH_2), 1.395 (p, 2H, J_{vic} 7.0 Hz, octyl CH_2), 1.340-1.210 (m, 8H, octyl CH_2), 0.873 (t, 3H, J_{vic} 6.9 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 138.59, 138.45, 138.04 (3 × aromatic quart.), 128.51, 128.40, 128.11, 127.93, 127.90, 127.70, 127.65 (aromatic CH), 84.55 (C-3), 82.35 (C-2), 77.66 (C-4), 75.70 (C-5), 75.10, 74.93, 74.89 (3 benzylic), 70.47 (octyl C_1), 61.79 (C-6), 31.83, 29.82, 29.41, 29.25, 26.16, 22.67 (octyl C_2-C_6), 14.10 (octyl CH_3): Anal. Calcd for $C_{35}H_{45}DO_6$ (563.76): C, 74.57; H, 8.40. Found: C, 74.59: H, 8.27.

(6S) - [6-^{2}H] Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**98S**).

A mixture of **96S** (67 mg, 0.119 mmol), silver trifluoromethanesulfonate (63 mg, 0.245 mmol), molecular sieves 4 Å (200 mg), collidine (13 µL, 98 µmol) and dry CH_2Cl_2 (4 mL) was cooled to -78 °C. To the resulting mixture was added dropwise a solution of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl bromide **75** [123] (120 mg, 0.213 mmol) in dry CH_2Cl_2 (1 mL) under argon. The mixture was allowed to warm to r.t. within 1 h. After addition of excess tetraethylammonium chloride (41 mg, 0.25 mmol), the

mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, saturated $NaHCO_3$ and water, and then dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography using toluene-EtOAc (10:1) as eluant to provide compound **98S** (119 mg, 97%) as a syrup; $[\alpha]_D$ +18.5° (*c* 0.3, CH_2Cl_2); ^1H NMR (360 MHz, $CDCl_3$): δ 7.40-7.10 (m, 30H, Ar-H), 5.428 (dd, 1H, $J_{1',2'}$ 1.9 Hz, $J_{2',3'}$ 2.9 Hz, H-2'), 4.962, 4.955, 4.855, 4.854, 4.775, 4.723, 4.692, 4.655, 4.520, 4.500, 4.450, 4.430 (12d, each 1H, J_{gem} 11.0 Hz, $PhCH_2$), 4.901 (d, 1H, H-1'), 4.353 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 3.939 (dd, 1H, $J_{3',4'}$ 9.2 Hz, H-3'), 3.873 (dd, 1H, $J_{4',5'}$ 9.6 Hz, H-4'), 3.788 (ddd, 1H, $J_{5',6'a}$ 4.0 Hz, $J_{5',6'b}$ 1.6 Hz, H-5'), 3.771 (d, 1H, $J_{5,6R}$ 4.7 Hz, H-6*pro*R), 3.670 (dd, 1H, J_{gem} 11.0 Hz, $J_{5',6'a}$ 4.0 Hz, H-6'a), 3.590 (dd, 1H, $J_{5',6'b}$ 1.6 Hz, H-6'b), 2.142 (s, 3H, Ac), 0.865 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3). ^{13}C NMR (100.6 MHz): δ 170.35 (C=O), 138.61, 138.55, 138.49, 138.23, 138.02, 137.86 (aromatic quart.), 128.36, 128.25, 128.16, 127.87, 127.75, 127.67, 127.64, 127.51, 127.40 (aromatic CH), 103.52 (C-1), 97.78 (C-1'), 75.66, 74.97, 74.88, 74.77, 73.27, 71.61 (6 × benzylic), 70.08 (octyl C_1), 65.81 (C-6), 31.82, 29.72, 29.43, 29.25, 26.20, 22.66 (octyl C_2-C_7), 21.12 (Ac), 14.08 (octyl CH_3); Anal. Calcd for $C_{64}H_{75}DO_{12}$ (1038.32): C, 74.03; H, 7.47. Found: C, 74.02; H, 7.33.

(6R) - [6-^{2}H] Octyl 2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**98R**).

The same procedure as that described for the preparation of **98S** was applied for the preparation of **98R** with **96R** (85 mg, 0.151 mmol), **75** [123] (151 mg, 0.272 mmol), silver trifluoromethanesulfonate (78 mg, 0.302 mmol), collidine (16 µl, 0.121 mmol), molecular sieves 4 Å (300 mg) and CH_2Cl_2 (8 mL) to give **98R** (146 mg, 93%) as a syrup; $[\alpha]_D$ +21.75° (*c* 0.40, CH_2Cl_2); ^1H NMR (360 MHz, $CDCl_3$): δ 3.671 (d, 1H, $J_{5,6S}$ 1.4 Hz, H-6*pro*S), other signals of ^1H NMR and ^{13}C NMR are identical with those

of **98S**. Anal. Calcd for $C_{64}H_{75}DO_{12}$ (1038.32): C, 74.03; H, 7.47. Found: C, 74.38; H, 7.85.

(6S) - [6-^{2}H] Octyl 3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**99S**).

Compound **98S** (100 mg, 96 μmol) was treated with methanolic NaOMe (0.05 N, 10 mL) at r.t. for 10 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left a residue that was purified by column chromatography (toluene-EtOAc 10:1) to give **99S** as a white solid (95 mg, 99%); ^{1}H NMR ($CDCl_3$, 360 MHz): δ 7.40-7.10 (m, 30H, Ar-H), 4.992 (d, 1H, $J_{1',2'}$ 1.8 Hz, H-1'), 4,677 (d, 1H, $J_{1'',2''}$ 7.8 Hz, H-1''), 4.358 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.092 (ddd, 1H, $J_{2',3'}$ 4.5 Hz, H-2'), 3.895 (dt, 1H, J_{gem} 9.5 Hz, J_{vic} 6.6 Hz, octyl C_1-H_a), 3.817 (d, 1H, $J_{5,6R}$ 4.8 Hz, H-6*pro*R), 3.465 (dt, 1H, octyl C_1-H_b), 3.390 (dd, 1H, $J_{4,5}$ 9.1Hz, $J_{5,6R}$ 4.8 Hz, H-5), 2.370 (d, 1H, $J_{2',OH}$ 3.0 Hz, OH-2'), 0.873 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3).

(6R) - [6-^{2}H] Octyl 3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**99R**).

Compound **98R** (130 mg, 0.125 mmol) was treated in the same manner for the preparation of **99S** to give **99R** (118 mg, 95%) as a white solid; ^{1}H NMR ($CDCl_3$, 360 MHz): δ 3.385 (d, 1H, $J_{5,6S}$ 1.7 Hz, H-6*pro*S), 3.385 (dd, 1H, $J_{4,5}$ 9.1 Hz, $J_{5,6S}$ 1.7 Hz, H-5), 2.397 (d, 1H, $J_{2',OH}$ 2.8 Hz, C2'-OH), other signals of ^{1}H NMR are identical with those of **99S**.

O-(3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-trichloroacetimidate (**100**).

To a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-glucopyranosyl bromide (**14**) (1.5 g, 3.01 mmol) [120] in acetone (10 mL) at 0 °C was added water (0.2 mL) and silver carbonate (1.0 g, 3.62 mmol). The reaction mixture was vigorously stirred, then warmed to r.t. and stirred overnight (10 hrs). The reaction mixture was filtered through a Celite bed which was washed with acetone. The acetone filtrate was concentrated, and residue was dissolved in CH_2Cl_2 and washed with water. The CH_2Cl_2 solution was dried ($MgSO_4$) and concentrated. The residue was recrystallized in methanol to give the hemiacetal (980 mg, 75%) as a intermediate. The intermediate was not characterized but was used directly for the preparation of imidate **100**. To a solution of the hemiacetal intermediate (820 mg, 1.88 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C was added DBU (28 μL, 0.188 mmol) and trichloroacetonitrile (700 μL). The reaction mixture was stirred at r.t. for 2 hrs and was concentrated. The residue was purified by chromatography using hexane-ether-Et_3N (50:50:1) as eluant to afford imidate **100** as a syrup (661 mg, 61%); ^1H NMR (360 MHz, $CDCl_3$): δ 8.480 (s, 1H, NH), 7.843 (dd, 2H, J 5.5, 3.2 Hz, phthalimido C3-H, C6-H), 7.728 (d, 2H, J 5.5, 3.2 Hz, phthalimido C4-H, C5-H), 6.630 (d, 1H, $J_{1,2}$ 9.0 Hz, H-1), 5.923 (dd, 1H, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 9.5 Hz, H-3), 5.286 ($J_{4,5}$ 10.0 Hz, H-4), 4.641 (dd, 1H, H-2), 4.400 (dd, 1H, $J_{5,6a}$ 4.8 Hz, J_{gem} 12.3 Hz, H-6a), 4.210 (dd, 1H, $J_{5,6b}$ 2.2 Hz, H-6b), 4.090 (ddd, 1H, H-5), 2.127, 2.054, 1.898 (3s, each 3H, 3 × Ac).

(6S) - [6-^{2}H] Octyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**101S**).

A mixture of **99S** (90 mg, 90.3 μmol), AgOTf (58 mg, 0.223 mmol), molecular sieves 4 Å (100 mg), collidine (9.5 μL, 72.2 μmol) and dry CH_2Cl_2 (4 mL) was cooled to -78 °C. To the resulting mixture was added dropwise a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-glucopyranosyl bromide (**14**) [120] (90 mg, 0.181 mmol) in dry dichloromethane (1 mL) at -40 °C under argon. The mixture was allowed to warm to room temperature within 1 h. After stirring for 3 hrs, excess tetraethylammonium chloride (31 mg, 0.19 mmol) was added, and the mixture was stirred for another 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, sat. $NaHCO_3$ and water, and then dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography using hexane-EtOAc (2:1) as eluant to provide compound **101S** as a syrup (40 mg, 31%). $[\alpha]_D$ -1.6° (*c* 0.3, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 360 MHz): 7.890-7.520 (m, 4H, phthalimido Ar-H), 7.380-7.020 (m, 30H, benzyl Ar-H), 5.804 (dd, 1H, $J_{2",3"}$ 10.8 Hz, $J_{3",4"}$ 9.1 Hz, H-3"), 5.561 (d, 1H, $J_{1",2"}$ 9.8 Hz, H-1"), 5.215 (dd, 1H, $J_{4",5"}$ 9.4 Hz, H-4"), 4.962 (d, 1H, J_{gem} 11.2 Hz, $PhCH_2$), 4.936 (d, 1H, J_{gem} 11.3 Hz, $PhCH_2$), 4.823 (d, 1H, J_{gem} 11.1 Hz, $PhCH_2$), 4.750 (d, 2H, J_{gem} 11.1 Hz, $PhCH_2$), 4.720 (d, 2H, J_{gem} 10.8 Hz, $PhCH_2$), 4.710 (d, 1H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.535 (d, 1H, J_{gem} 11.9 Hz, $PhCH_2$), 4.508 (dd, 1H, H-2"), 4.377 (d, 1H, $J_{1,2}$ 9.6 Hz, H-1), 4.254 (dd, 1H, J_{gem} 12.1 Hz, $J_{5",6"a}$ 2.1 Hz, H-6"a), 4.240 (dd, 1H, $J_{2',3'}$ 2.8 Hz, H-2'), 4.106 (dt, 1H, J_{gem} 9.5 Hz, J_{vic} 6.5 Hz, octyl C_1-H_a), 4.008 (ddd, $J_{5",6"b}$ 4.9 Hz, H-5"), 3.976 (s, 2H, $PhCH_2$), 3.790 (dd, 1H, $J_{3',4'}$ 8.6 Hz, H-3'), 3.757 (d, 1H, $J_{5,6R}$ 3.7 Hz, H-6*pro*R), 3.324 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-5), 2.071, 2.045, 1.864 (3s, each 3H, 3 × Ac), 0.868 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 170.71, 170.14, 169.42 (C=O), 138.62, 138.54, 138.41, 138.38, 138.02, 137.94 (aromatic quart.), 133.94 (phthalimido C3, C6), 128.34 (phthalimido C1,

C2), 128.34, 128.31, 128.19, 128.14, 128.10, 128.03, 127.89, 127.73, 127.70, 127.60, 127.54, 127.33, 127.23 (aromatic methine), 123.39 (phthalimido C4, C5), 103.86 (C-1), 97.64 (C-1'), 96.27 (C-1"), 75.71, 74.76, 74.74, 72.58, 70.66, 69.48 ($PhCH_2$), 70.63 (octyl C1), 66.03 (C-6), 62.38 (C-6', C-6"), 54.36 (C-2"), 31.83, 29.74, 29.47, 29.25, 26.18, 22.64 (octyl C2-C7), 20.78, 20.62, 20.45 (3 × Ac), 14.07 (octyl CH_3); Anal. Calcd for $C_{82}H_{92}DO_{20}N$(1413.66): C, 69.67; H, 6.70; N, 0.99. Found: C, 69.70; H, 6.41; N, 1.19.

(6R) - [6-^{2}H] Octyl 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**101R**).

A mixture of **99R** (98 mg, 98.4 μmol), **100** (87 mg, 969 μmol), powdered molecular sieves 4 Å (500 mg) and CH_2Cl_2 (8 mL) was stirred at r.t. for 30 min and cooled to 0 °C. To the mixture was added dropwise a solution of TMSOTf (19 μL, 97 μmol) in CH_2Cl_2 (1 mL) under argon. The reaction mixture was warmed to r.t. within 30 min. After stirring at r.t. for 2 hrs, Et_3N (0.4 mL) was added and the mixture was filtered through Celite and concentrated. The residue was purified by chromatography using toluene-EtOAc (4:1) as eluant to afford **101R** (125 mg, 90%) as a syrup; $[\alpha]_D$ -2.1° (*c* 0.5, CH_2Cl_2); ^{1}H NMR (360 MHz, $CDCl_3$): δ 3.562 (d, 1H, $J_{5,6S}$ 1.7 Hz, H-6*pro*S), 3.332 (dd, 1H, $J_{4,5}$ 9.5 Hz, $J_{5,6S}$ 1.7 Hz, H-5), other signals of ^{1}H NMR and ^{13}C NMR are identical with those of **101S**. Anal. Calcd for $C_{82}H_{92}DO_{20}N$ (1413.66): C, 69.67; H, 6.70; N, 0.99. Found: C, 69.94; H, 6.56; N, 1.10.

(6S) - [6-^{2}H] Octyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**102S**).

A solution of **101S** (28.5 mg, 20.2 μmol) in *n*-butanol (5 mL) was added to ethylenediamine (1 mL). The reaction mixture was stirred at 70 °C for 5 hrs. Concentration was followed by addition and evaporation twice of toluene followed by methanol. The residue was not characterized but was dissolved in dry pyridine (1 mL) to which acetic anhydride (0.5 mL) was added. After stirring for 4 hrs at r.t., the solution was concentrated. The residue was purified by column chromatography using hexane-EtOAc (1:1) as eluant, to give **102S** as a colorless syrup (18 mg, 69% in two steps). $[\alpha]_D$ +10.5° (*c* 0.75, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 360 MHz): δ 5.694 (d, 1H, $J_{2",3"}$ 10.5 Hz, H-3"), 5.534 (d, 1H, $J_{1",2"}$ 7.5 Hz, H-1"), 5.134 (d, 1H, $J_{1,2}$ 8.3 Hz, H-1), 5.020 (dd, 1H, $J_{4",5"}$ 9.6 Hz, H-4"), 4.817 (d, 1H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.293 (dd, 1H, J_{gem} 12.3 Hz, $J_{5",6"a}$ 5.1 Hz, H-6"a), 4.216 (dd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.182 (dd, 1H, $J_{5",6"b}$ 2.2 Hz, H-6"b), 3.887 (dd, 1H, $J_{3',4'}$ 9.0 Hz, H-3'), 3.853 (d, 1H, $J_{5,6R}$ 4.1 Hz, H-6*pro*R), 3.827 (ddd, 1H, H-5"), 3.378 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-5), 2.049, 2.025, 2.004, 1.756 (4s, each 3H, 4 × Ac), 0.878 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 171.30, 170.74, 170.19, 169.67 (C=O), 138.80, 138.57, 138.45, 138.44, 137.98 (aromatic quart.), 128.42, 128.24, 128.11, 127.97, 127.85, 127.81, 127.74, 127.70, 127.60, 127.57, 127.46 (aromatic methine), 103.92 (C-1), 97.76 (C-1'), 97.67 (C-1"), 70.71 (octyl C1), 66.03 (C-6), 62.56 (C-6', C-6"), 56.26 (C-2"), 31.89, 29.79, 29.51, 29.31, 26.22, 22.70 (octyl C2-C7), 23.26, 20.82, 20.76, 20.72 ($\underline{CH_3}CO$), 14.13 (octyl CH_3); Anal. Calcd for $C_{76}H_{92}DO_{19}N$ (1325.48): C, 68.87; H, 7.15; N, 1.06. Found: C, 68.80; H, 6.95; N, 1.06.

(6R) - [6-^{2}H] Octyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**102R**).

A solution of **101R** (122 mg, 86.3 μmol) in butanol (15 mL) was added to ethylenediamine (3 mL). The resulting mixture was processed in the same way for the preparation of **102S** to afford **102R** (109 mg, 95%) as a syrup. $[\alpha]_D$ +9.8° (*c* 0.8, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 360 MHz): δ 3.622 (d, 1H, $J_{5,6S}$ 1.4 Hz, H-6*pro*S), 3.370 (dd, 1H, $J_{4,5}$ 9.5 Hz, H-5), other signals of ^{1}H NMR and ^{13}C NMR are identical with those of **102S**. Anal. Calcd for $C_{76}H_{92}DO_{19}N$ (1325.48): C, 68.87; H, 7.15; N, 1.06. Found: C, 69.12; H, 6.97; N, 1.28.

(6S) - [6-^{2}H] Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**5S**).

Compound **102S** (17 mg, 12.8 μmol) was treated with methanolic NaOMe (0.05 N, 1 mL) at r.t. for 4 hrs. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left a residue. The residue was not characterized, but was dissolved in methanol (5 mL) containing 5% palladium hydroxide on charcoal (50 mg). The mixture was stirred under one atmosphere H_2 for 4 hrs. Removal of the catalyst by filtration followed by concentration left a residue which was purified by chromatography on Iatrobeads using CH_2Cl_2-MeOH-H_2O (65:35:6) as eluant to give a glassy material. This material was adsorbed onto a Sep-Pak C-18 cartridge in water, the cartridge was washed with water (10 mL) and eluted with HPLC grade methanol (10 mL). Evaporation of the solvent, dissolution of the residue in water, filtration through a Millex filter and lyophilization of the filtrate gave **5S** (6.0 mg, 71% in two steps) as a white powder; $[\alpha]_D$ -7.2° (*c* 0.5, CH_3OH); ^{1}H NMR (500 MHz, D_2O): δ 4.899 (d, 1H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.570 (d, 1H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.456 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 4.113 (dd, 1H, $J_{2',3'}$ 3.1 Hz, H-2'), 3.936 (d, 1H, $J_{5,6R}$ 4.9 Hz, H-6*pro*R), 3.916 (dd,

1H, J_{gem} 12.3 Hz, $J_{5",6"a}$ 2.3 Hz, H-6"a), 3.898 (dd, 1H, J_{gem} 11.9 Hz, $J_{5',6'a}$ 1.8 Hz, H-6'a), 3.888 (dt, 1H, J_{gem} 10.0 Hz, J_{vic} 6.5 Hz, octyl C1-H_a), 3.834 (dd, 1H, $J_{3',4'}$ 9.6 Hz, H-3'), 3.765 (dd, 1H, $J_{5",6"b}$ 5.4 Hz, H-6"b), 3.705 (dd, 1H, $J_{2",3"}$ 10.2 Hz, H-2"), 3.645 (dt, 1H, octyl, C1-H_b), 3.630 (dd, 1H, $J_{5',6'b}$ 7.5 Hz, H-6'b), 3.582 (dd, 1H, $J_{4,5}$ 9.8 Hz, H-5), 3.575 ($J_{4',5'}$ 9.6 Hz, H-5'), 3.562 (dd, 1H, $J_{3",4"}$ 9.5 Hz, H-3"), 3.524 (dd, 1H, H-4'), 3.482 (dd, 1H, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 9.6 Hz, H-3), 3.471 (dd, 1H, $J_{4,5}$ 9.6 Hz, H-4"), 3.468 (dd, 1H, H-4), 3.432 (ddd, 1H, H-5"), 3.285 (dd, 1H, H-2), 2.062 (s, 3H, Ac), 1.624 (p, 2H, J_{vic} 7.0 Hz, octyl CH_2), 1.380-1.260 (m, 10H, octyl $5 \times CH_2$), 0.865 (t, 3H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 175.67 (C=O), 103.19 (C-1), 100.38 (C-1"), 97.66 (C-1'), 77.10 (C-2'), 76.88 (C-3), 76.70 (C-5"), 74.87 (C-5), 74.21 (C-5'), 73.95 (C-2), 73.71 (C-3"), 71.67 (octyl C_1), 70.75 (C-4"), 70.47 (C-3'), 70.30 (C-4), 68.08 (C-4'), 66.28 (C-6), 62.39 (C-6'), 61.46 (C-6"), 56.24 (C-2"), 31.94, 29.62, 29.28, 29.21, 25.89, 22.87 (octyl C_2-C_7), 23.18 (Ac), 14.27 (octyl CH_3); FAB MS: m/z 697.0 $(M+K)^+$, 681.0 $(M+Na)^+$; Exact FAB MS for $C_{28}H_{50}DNO_{16}Na$, theoretical MS: 681.3168; Found: 681.3156 (std. deviation 1.9 PPM).

(6S) - [6-^{2}H] Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**5R**).

Compound **102R** (76 mg, 57.3 μmol) was treated with the same procedure for the preparation of **5S** to afford **5R** (30 mg, 80%) as a white powder. ^1H NMR (500 MHz, D_2O): δ 3.740 (d, 1H, $J_{5,6S}$ 2.0 Hz, H-6*pro*S), 3.581 (dd, 1H, $J_{4,5}$ 9.8 Hz, H-5), other signals of ^1H NMR and ^{13}C NMR are identical with those of **5S**; FAB MS: m/z 697.1 $(M+K)^+$, 681.2 $(M+Na)^+$, 659.1 $(M+H)^+$; Exact FAB MS for $C_{28}H_{50}DNO_{16}Na$, theoretical MS: 681.3168; Found: 681.3147 (std. deviation 3.1 PPM).

CHAPTER 7

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CHAPTER 8

Appendix: Enzyme Kinetics

Enzymes [167] are specific proteins which mediate almost all biochemical reactions. The striking characteristics of enzymes are their extraordinary catalytic power and high specificity. Enzymes are highly specific both in the reaction catalyzed and in the recognition of their reactants called substrates. The specific region of an enzyme which binds to the substrate and catalyzes the reaction is called the active site. There are binding groups and catalytic groups in the active sites of enzymes.

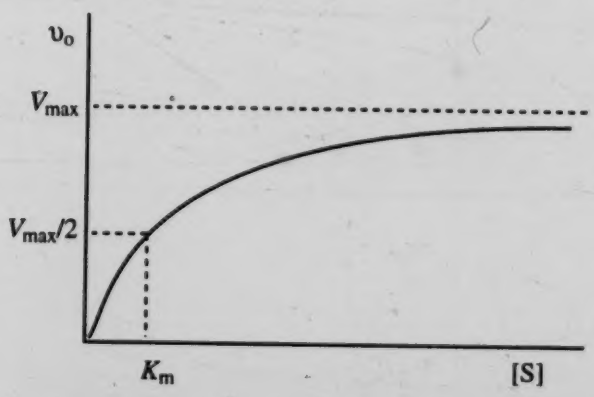

Fig. 52. A plot of the initial velocity υ_o of a simple Michaelis-Menten reaction versus the substrate concentration [S].

For many enzymes, the initial rate of reaction, υ_o, varies with the substrate concentration, [S], in a rectangular hyperbola manner which is shown in Fig. 52. In 1913, Leonor Michaelis and Maude Menten proposed a simple model to account for the kinetic properties of these enzymes:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

An enzyme (E) combines with a substrate (S) to form an enzyme-substrate complex (ES), with a rate constant k_1. The ES complex can dissociate back to E and S, with a rate constant k_{-1}, or it can proceed to form product (P), with a rate constant k_2.

The Michaelis-Menten equation is the basic equation of enzyme kinetics to describe a rectangular hyperbolic relation which is shown in Fig. 52.

$$\upsilon_o = \frac{V_{max}[S]}{[S] + K_m} \qquad (1)$$

V_{max} is the maximal velocity of the enzyme-catalyzed reaction. At a sufficiently high substrate concentration, the reaction rate reaches a maximum.

The Michaelis constant, K_m, is defined as: $K_m = (k_{-1}+k_2)/k_1$. K_m can also be operationally defined as the substrate concentration at which the reaction velocity is half-maximal. The magnitude of K_m varies with the identity of the enzyme and the nature of the substrate. It partially reflects the binding affinity of the enzyme towards its substrate. It is also a function of temperature and pH.

The Michaelis-Menten equation can be transformed into a double-reciprocal form [equation (2)] that gives a straight line plot to facilitate the analysis of kinetic data. Such a plot of $1/\upsilon_o$ versus 1/[S] (Lineweaver-Burk plot) yields a straight line with an intercept of $1/V_{max}$ on the y-axis, an intercept of $-1/K_m$ on the x-axis, and a slope of K_m/V_{max}. (Fig. 53.). However, kinetic parameters (K_m and V_{max}) can also be obtained by fitting the initial

rate data to the Michaelis-Menten equation using computer program such as Sigma Plot 4.10 [90].

$$\frac{1}{\upsilon_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \frac{1}{[S]} \qquad (2)$$

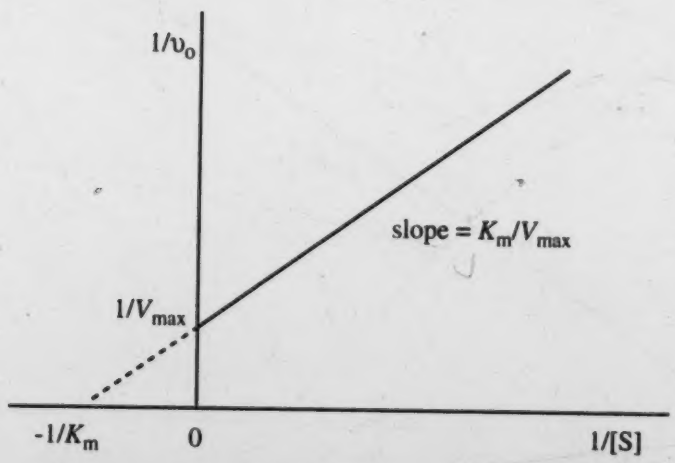

Fig. 53. A double-reciprocal (Lineweaver-Burk) plot of enzyme kinetics

Most enzymes are sensitive to inhibition by specific agents that interfere with the binding of a substrate at the active site or with the conversion of the enzyme-substrate complex into products. Enzyme inhibition can be either reversible or irreversible. In irreversible inhibition, the inhibitor becomes covalently bound to the enzyme or bound so tightly that its dissociation from the enzyme is very slow. In reversible inhibition, the inhibitor binds and dissociates with the enzyme in a rapid equilibrium.

A competitive inhibitor is a substance that resembles the substrate structurally and competes directly with a normal substrate for the binding site. A competitive inhibitor binds reversibly to the active site of the enzyme but differs from the substrate as it is not reactive.

The general model describing competitive inhibition is given by the following reaction scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$+$$

$$I$$

$$K_i \updownarrow$$

$$EI + S \longrightarrow \text{No Reaction}$$

Here it is assumed that I, the inhibitor, binds reversibly to the enzyme in a rapid equilibrium with a dissociation constant K_i = [E][I]/[EI]. K_i is also called the inhibitory constant, it reflects the binding affinity between the inhibitor and the enzyme.

In competitive inhibition, the binding between the substrate-enzyme and inhibitor-enzyme is mutually exclusive. At a sufficiently high substrate concentration, almost all the active sites are filled by substrates, and the enzyme is fully operative. Therefore, V_{max} is not altered by a competitive inhibitor. However, because the presence of a competitive inhibitor has the effect of making the substrate concentration [S] appears more dilute than it actually is, the K_m value (apparent K_m) appears larger than it really is (Fig. 54.).

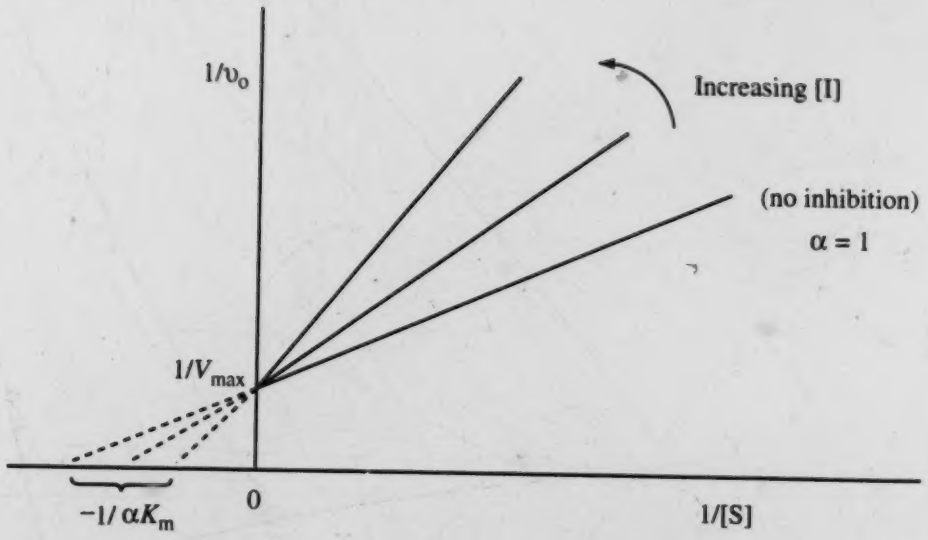

Fig. 54. A double-reciprocal plot of competitive inhibition

The velocity equation for competitive inhibition in reciprocal form is:

$$\frac{1}{\upsilon_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} \qquad (3)$$

$$\alpha = 1 + \frac{[I]}{K_i}$$

The velocity equation can also be expressed in terms of "percent inhibition" (i%) as follow [168]:

$$i = \frac{100\ [I]}{[I] + (1 + [S] / K_m)\ K_i} \qquad (4)$$